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MICROBIAL FERMENTATION AND BIOACTIVE COMPOUND ENHANCEMENT IN TRADITIONAL FRUIT-BASED FUNCTIONAL FOODS OF PAKISTAN

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Abstract

Traditional fruit-based fermented foods of Pakistan represent a rich intersection of cultural heritage, natural preservation, and functional nutrition. This review comprehensively analyzes the microbial fermentation processes and bioactive compound enhancement mechanisms in indigenous Pakistani fruit and botanical ferments, including Kanji, Anardana, Phalsa and Jamun beverages, Hunza Apricot Sirka, and Sea Buckthorn ferments. The spontaneous fermentation of these substrates is driven by complex microbial consortia predominantly lactic acid bacteria (Lactiplantibacillus plantarum, Loigolactobacillus coryniformis, Leuconostoc mesenteroides), yeasts (Saccharomyces cerevisiae), and acetic acid bacteria (Acetobacter aceti). These microorganisms orchestrate enzymatic hydrolysis of cell wall polysaccharides, biotransformation of high-molecular-weight polyphenols into bioavailable aglycones, conversion of ellagitannins to urolithin precursors, and degradation of anti-nutritional factors including phytates (78% reduction), oxalates, alkaloids, and glucosinolates. Such biochemical transformations substantially elevate total phenolic content, flavonoid content, and radical scavenging capacity (40–120% increase in DPPH activity), while imparting therapeutic properties including antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, hypoglycemic, and antiproliferative activities. Despite this potential, transitioning these artisanal ferments into standardized functional nutraceuticals faces challenges in process standardization, starter culture development, and regulatory compliance. This review examines industrial valorization strategies including defined starter inoculation, solid-state fermentation of fruit

pomace, and adherence to Pakistan Standards and Quality Control Authority (PSQCA) frameworks. The findings highlight the significant potential of traditional Pakistani fruit ferments as sustainable sources of functional foods and nutraceuticals, warranting further clinical validation and commercial development.

1. INTRODUCTION

Traditional fermented foods and beverages represent an intersection of cultural heritage, natural preservation, and functional nutrition. Across the diverse agro-ecological zones of Pakistan ranging from the fertile alluvial plains of Punjab and Sindh to the arid plateaus of Balochistan and the high-altitude alpine valleys of Gilgit-Baltistan indigenous communities have historically utilized spontaneous microbial fermentation to extend the shelf-life of seasonal fruits and botanicals (Saeed et al., 2025). Beyond simple preservation, empirical food preparation traditions have established bioprocessing methods that transform raw, highly perishable horticultural matrices into shelf-stable, therapeutic functional foods (Khan et al., 2025).

In recent years, functional food research has increasingly shifted toward understanding the precise biochemical mechanisms by which wild and cultivated microorganisms alter plant matrices. In fruit-based substrates, natural fermentation relies on complex microbial consortia primarily lactic acid bacteria (LAB), yeasts, and acetic acid bacteria (AAB) that systematically metabolize simple sugars, organic acids, and complex macromolecular structures (Mubeen et al., 2025). This metabolic activity generates primary and secondary metabolites, including short-chain fatty acids (SCFAs), organic acids, bacteriocins, and bioactive peptides, while inducing profound structural transformations in plant polyphenols (Alharbi et al., 2026).

The primary obstacle to the nutritional optimization of raw fruits and botanicals lies in the structural entrapment of polyphenols within complex cell wall polysaccharides (such as pectin, cellulose, and hemicellulose) and the co-occurrence of anti-nutritional factors (ANFs) such as phytates, oxalates, condensed tannins, and alkaloids. Raw fruits often exhibit limited bioavailability of their native antioxidants because high-molecular-weight polyphenols and glycosylated flavonoids cannot be efficiently absorbed across the human intestinal epithelium (Farid et al., 2026).

Microbial biotransformation during traditional Pakistani fermentations overcomes these bio-accessibility barriers through enzymatic hydrolysis. Microorganisms secrete extracellular glycosidases, esterases, cellulases, and tannases that cleave glycosidic bonds, break down plant cell walls, and convert complex phenolic glycosides and ellagitannins into unesterified aglycones and low-molecular-weight phenolics. This enzymatic unlocking dramatically increases total phenolic content (TPC), total flavonoid content (TFC), and free radical scavenging capacity (Doriya et al., 2022).

Furthermore, microbial metabolic pathways actively degrade anti-nutritional compounds, enhancing trace mineral solubility (Ca^{2+}, Fe^{2+}, Zn^{2+}) and overall protein digestibility. This review provides a comprehensive analysis of traditional fruit-based and botanical fermented foods in Pakistan, characterizing their microbial ecology, detailing the biochemical pathways of bioactive enhancement, evaluating their therapeutic potential, and examining the industrial and regulatory frameworks necessary to transition these artisanal ferments into standardized functional nutraceuticals (Gunawardena et al, 2024).

2. Spectrum of Traditional Fruit and Botanical Fermented Foods in Pakistan

Pakistan's traditional fermented landscape encompasses a rich variety of fruit- and botanical-based preparations, each adapted to regional fruit availability, climatic conditions, and traditional knowledge systems. These range from naturally fermented solar beverages in the central plains to high-altitude stone-fruit ferments in the northern mountain ranges (Wah et al., 2023).

The bioprocessing cascade typically initiates with harvested raw horticultural substrates or wild botanicals, such as black carrots, beetroot, sour pomegranates, Phalsa, Jamun berries, sea buckthorn, and apricots. These plant substrates undergo spontaneous or solid-state fermentation initiated by indigenous epiphytic microflora, crushed mustard seeds, or back-slopping techniques (Jabeen et al., 2024).

During this process, a dynamic microbial succession occurs wherein adapted strains of *Lactiplantibacillus plantarum*, *Loigolactobacillus coryniformis*, *Saccharomyces cerevisiae*, and *Acetobacter* species sequentially drive the metabolic biotransformation of the plant matrix. This metabolic cascade induces enzymatic cleavage of phenolic glycosides, hydrolyzes complex tannins into ellagic acid, degrades cell wall structures, and breaks down anti-nutritional factors, ultimately yielding bio-enriched functional foods with elevated radical scavenging capacity and probiotic viability (Abbasi et al., 2013).

Kanji: The Solar-Fermented Botanical Drink

Among the most widely documented traditional fermented functional beverages in Punjab and neighboring regions is Kanji, an acidic, dark-purple, naturally fermented drink prepared primarily during the late winter and early spring months. The classical recipe utilizes deep purple or black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens*), often supplemented with red beetroot (*Beta vulgaris*) to intensify color and sugar content (Joshi, 2016).

The roots are washed, peeled, cut into uniform batons, and submerged in cooled, previously boiled water inside sterile earthen (*matkas*) or glass vessels. Crushed mustard seeds either black mustard (*Brassica nigra*) or yellow mustard (*Sinapis alba*) along with red chili powder (*Capsicum annuum*), black salt (*Kala Namak*), and occasionally small quantities of sucrose are added to the brine. The mustard seeds serve not only as a spice but as a major source of indigenous microbial inocula and glucosinolates (Zarwa et al., 2026).

The jar is covered loosely with cheesecloth or a lid and placed in direct sunlight for 2 to 7 days, depending on ambient solar irradiation and temperature. Solar heating maintains a micro-warm environment that accelerates the growth of native heterofermentative and homofermentative LAB, predominantly *Lactiplantibacillus plantarum* and *Leuconostoc* species (Khan & Shaukat, 2006).

As lactic acid accumulates, the pH drops rapidly from approximately 6.0 to below 3.5, imparting a sour, pungent, and refreshing taste. Simultaneously, anthocyanins (predominantly cyanidin-3-glucoside derivatives) from the black carrots and betalains from the beetroot are extracted and stabilized within the acidic medium, creating an antioxidant-rich probiotic beverage utilized traditionally for digestive ailments, hepatic cooling, and appetite stimulation (Akhtar et al., 2013).

Anardana and Pomegranate Biotransformations

Pomegranate (*Punica granatum* L.), locally known as *Anar*, is cultivated extensively across Balochistan, Khyber Pakhtunkhwa, and Punjab. While fresh arils are consumed directly, wild sour pomegranates (*Daru*) are traditionally processed into *Anardana* a partially fermented, sun-dried condiment used in Pakistani culinary and medicinal applications (Mansoor et al., 2023).

The extraction and slow solar-drying of the seeds encourage controlled surface fermentation by epiphytic yeast and LAB species. This process transforms the astringent, tannin-heavy pulp into a tangy, bio-enriched constituent rich in organic acids (citric, malic, and oxalic acids) and phenolic compounds (Konczak, 2004).

Additionally, liquid-state fermentations of whole pomegranate juice and pomegranate peel (*Punica granatum* peel) extracts using indigenous starter cultures have emerged as potent sources of low-molecular-weight phenolics. The high initial concentration of ellagitannins, particularly punicalagin isomers, undergoes microbial hydrolysis during fermentation, yielding free ellagic acid, gallic acid, and down-stream urolithin precursors with enhanced anti-inflammatory and neuroprotective activities (Centofanti et al., 2018).

Indigenous Berry Ferments: Phalsa and Jamun

Phalsa (*Grewia asiatica* Linn.) and Jamun (*Syzygium cumini* or *Eugenia jambolana* Linn.) are indigenous berries widely distributed throughout the tropical and subtropical plains of Pakistan. Both fruits possess extremely short fresh shelf-lives (24–48 hours) due to rapid post-harvest respiration and fungal decay, making them primary candidates for traditional microbial fermentation (Kamran et al., 2025).

Phalsa berries contain high levels of anthocyanins, quercetin, and pelargonidin, but their utilization is restricted by high titratable acidity and astringency. Traditional back-slopping and spontaneous anaerobic fermentation of Phalsa pulp convert harsh organic acids into smooth esters while liberating bound

flavonoids, transforming the raw fruit into anti-inflammatory and anticancer elixirs (Sidhu, J. S., & Zafar, 2019).

Similarly, Jamun fruit pulp and seeds are rich in anthocyanins (myricetin, delphinidin), gallic acid, and ellagic acid, possessing marked hypoglycemic and antioxidant properties. Fermentation of Jamun juice using strain-specific *Lactiplantibacillus plantarum* cultures degrades complex polysaccharides, reduces anti-nutritional factors, and yields functional vinegars (*Jamun Sirka*) and probiotic beverages that modulate blood glucose levels and reduce systemic oxidative stress (Sundar et al., 2024).

High-Altitude Ferments of Gilgit-Baltistan: Apricots, Sea Buckthorn, and Mulberries

In the northern high-altitude regions of Gilgit-Baltistan (including the Hunza, Nagar, and Skardu valleys), unique climatic conditions and isolated traditional knowledge have yielded specialized stone-fruit and berry ferments. Apricot (*Prunus armeniaca* L.) orchards yield vast surpluses during brief harvest windows (Abbas et al., 2021).

Indigenous populations prepare *Apricot Sirka* (fermented vinegar) and traditional fruit spirits such as *Hunza Apricot Arak* through a two-stage fermentation process. In the initial stage, natural yeasts (*Saccharomyces* spp.) convert apricot sugars into ethanol, followed by secondary acetous oxidation by *Acetobacter* species to produce acetic acid and aromatic esters (Sharma et al., 2025).

Sea Buckthorn (*Hippophae rhamnoides* L.), locally known as *Tsuk*, grows wild across the high-altitude riverbanks of Gilgit-Baltistan. The berries are exceptionally rich in vitamin C (ascorbic acid), carotenoids (β-carotene), and omega fatty acids, but possess intense acidity and astringency (Khan & Shaukat, 2006). Fermentation of Sea Buckthorn juice using single or co-culture lactic acid bacteria (e.g., *Lactiplantibacillus plantarum* co-cultured with *Lactobacillus acidophilus*) reduces harsh malic acid levels, synthesizes β-glucans, and stabilizes carotenoid esters, creating high-value functional beverages with cytotoxic potential against transformed liver cells (Rasul et al., 2019).

Table 1: Traditional Fruit-Based and Botanical Fermented Foods and Beverages in Pakistan

Product Name	Primary Raw Substrate	Key Microorganisms Involved	Key Bioactive Compounds Enhanced	Functional / Health Attributes	Regional Origin
Kanji	Black/Red Carrots (*Daucus carota*), Beetroot (*Beta vulgaris*), Mustard Seeds	*Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, Epiphytic Yeasts	Cyanidin-3-glucoside, Betalains, Free Phenolics, Glucosinolates	Probiotic gut health, Digestive aid, Liver cooling, Free radical scavenging	Punjab, Sindh
Anardana / Pomegranate Ferment	Sour Wild Pomegranate (*Punica granatum*) Arils & Peel	*Lactiplantibacillus* spp., *Saccharomyces* spp., Epiphytic Molds	Ellagic Acid, Gallic Acid, Punicalagin derivatives, Urolithin precursors	Anti-inflammatory, Anti-cholinesterase, Hepatoprotective, Neuroprotective	Balochistan, KPK, Punjab
Phalsa Ferment / Beverage	Phalsa Berry Pulp (*Grewia asiatica* Linn.)	*Lactiplantibacillus plantarum*, *Loigolactobacillus coryniformis*	Quercetin, Pelargonidin, Aglycone Flavonoids, Organic Acids	Anti-inflammatory, Antiproliferative, Antioxidant	Punjab, Sindh

Jamun Sirka / Probiotic Drink	Jamun Fruit Pulp & Seeds (*Syzygium cumini*)	*Lactiplantibacillus plantarum*, *Acetobacter aceti*	Myricetin, Delphinidin, Ellagic Acid, Ferulic Acid	Hypoglycemic, Anti-diabetic, Anti-microbial, Vascular protection	Punjab, KPK
Hunza Apricot Sirka / Arak	Apricot Fruit Pulp (*Prunus armeniaca*)	*Saccharomyces cerevisiae*, *Acetobacter aceti*, Wild Yeasts	Acetic Acid, Chlorogenic Acid, Aromatic Esters, Carotenoids	Digestive regulation, Antimicrobial, Metabolic stimulation	Gilgit-Baltistan
Sea Buckthorn Ferment	Sea Buckthorn Berries (*Hippophae rhamnoides*)	*Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*	Ascorbic Acid, β-Carotene, β-Glucans, Carotenoid Esters	Cytotoxic against HepG2, Immune modulation, High antioxidant power	Gilgit-Baltistan

3. Microbiological Profiles and Starter Dynamics

The spontaneous fermentation of fruit and botanical matrices relies on a complex succession of indigenous microorganisms. Understanding these microbial dynamics is essential for transitioning from highly variable household preparations to standardized industrial bioprocesses (Huang et al., 2017).

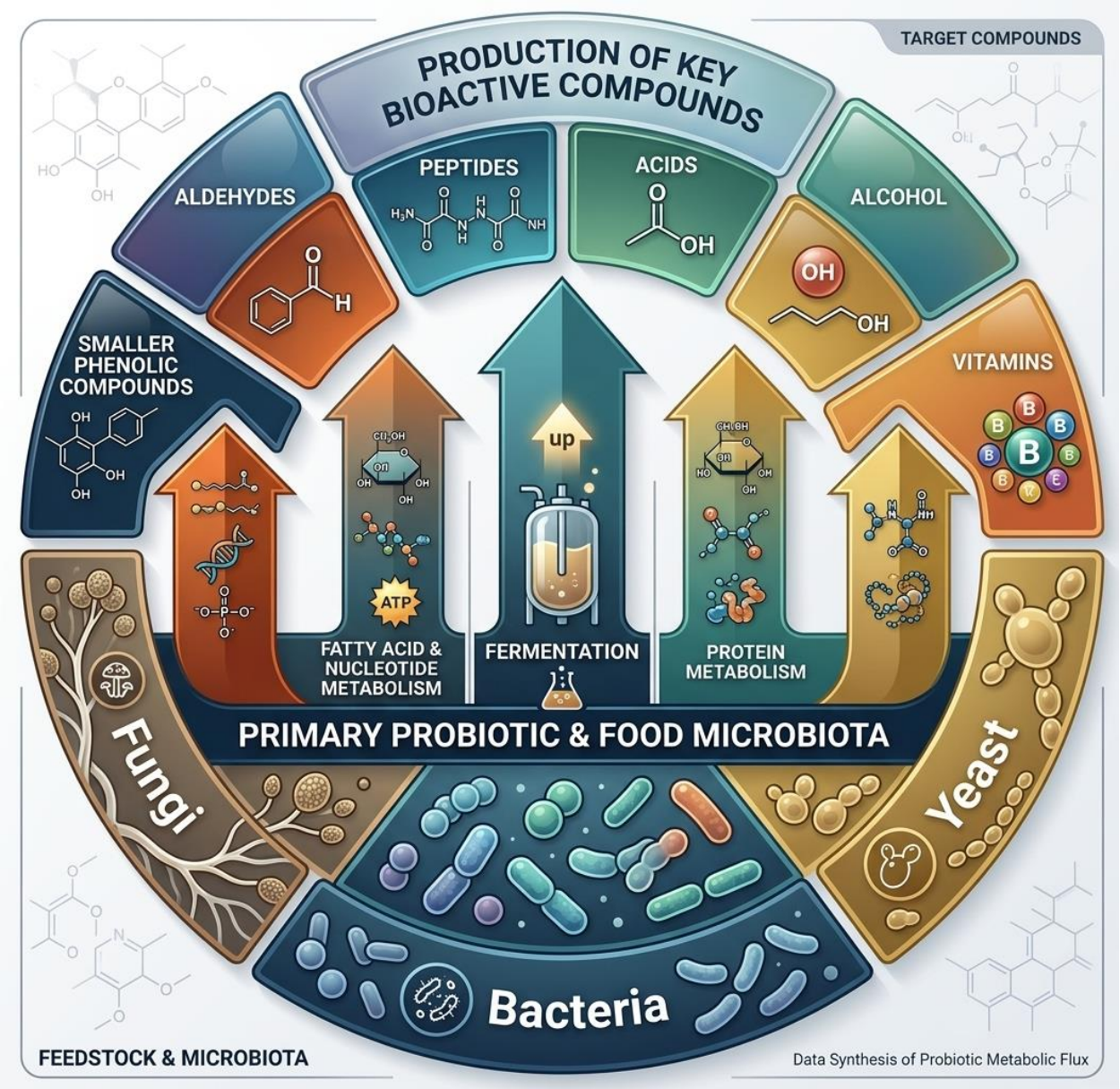


Figure 1: Probiotic Microbial Consortia and Metabolic Flux in Bioactive Compound Production During Plant Fermentation

Lactic Acid Bacteria (LAB)

Lactic Acid Bacteria constitute the predominant functional group across most Pakistani plant ferments. The primary isolates belong to the genera *Lactiplantibacillus* (formerly *Lactobacillus*), *Loigolactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella* (Naidu et al., 1999).

Lactiplantibacillus plantarum (e.g., strains 022AE, ATCC 8014) represents the most versatile and robust species isolated from Pakistani ferments. Genome sequencing of *L. plantarum* 022AE reveals a 3.23 Mb scaffold containing extensive genetic determinants for acid tolerance, bile salt hydrolase (*BSH*) activity, mucin adhesion, and the production of plantaricing bacteriocins (Axelsson, 2004).

Loigolactobacillus coryniformis (strain BCH-4) is another key organism isolated from indigenous ferments, known for its ability to produce potent antimicrobial secondary metabolites, including bis-(2-ethylhexyl) phthalate and cyclic dipeptides that inhibit fungal growth and detoxify aflatoxins (Raman et al., 2022).

During the initial hours of fermentation, heterofermentative species such as *Leuconostoc mesenteroides* dominate, metabolizing glucose via the phosphoketolase pathway to yield lactic acid, ethanol, carbon dioxide, and diacetyl, establishing anaerobiosis and early flavor profile. As acidity increases,

homofermentative strains like *L. plantarum* take over, utilizing glycolysis to rapidly produce lactic acid, driving the pH down to < 3.8 (Hayek et al., 2013).

Yeasts and Acetic Acid Bacteria (AAB)

Yeast species play a pivotal role in high-sugar fruit matrices such as apricots, pomegranates, and berries. *Saccharomyces cerevisiae* is the dominant ethanol producer, converting glucose and fructose into ethanol and carbon dioxide. However, non-*Saccharomyces* yeasts, such as *Torulaspora delbrueckii*, *Starmerella bombicola*, and *Wickerhamomyces saturnus*, contribute significantly to aromatic complexity by synthesizing isoamyl acetate, 2-phenylethyl acetate, and terpene alcohols (Raspor, P., & Goranovič , 2008).

In acetous ferments like *Apricot Sirka* and *Jamun Sirka*, Acetic Acid Bacteria primarily *Acetobacter aceti* and *Acetobacter pasteurianus* oxidize yeast-derived ethanol into acetic acid via sequential alcohol dehydrogenase (ADH) and aldehyde dehydrogenase ($ALDH$) membrane-bound enzyme systems (Han et al., 2024).

Fermentation Kinetics and Pathogen Exclusion

The metabolic activity during fruit fermentation induces rapid kinetic shifts in the substrate. During the initial 24 hours of fermentation, epiphytic yeasts and heterofermentative lactic acid bacteria initiate sugar metabolism from an initial pH of 5.5 to 6.0, generating carbon dioxide, minor ethanol, and early organic acids that reduce the pH to approximately 4.5 (Petka & Walczycka, 2026).

Between 24 and 72 hours, acid-tolerant homofermentative strains, specifically *Lactiplantibacillus plantarum*, dominate the system. The rapid accumulation of lactic acid ($pK_a = 3.86$) and acetic acid ($pK_a = 4.76$) drives the system pH below 3.5. In their lipophilic, undissociated forms, these organic acids freely penetrate the outer cell membranes of pathogenic and spoilage microorganisms (Hashemi et al., 2023).

Once inside the neutral cytoplasm, the acids dissociate, releasing proton ions (H^+) that collapse the transmembrane electrical potential, acidify the cytoplasm, and cause structural cell lysis. In vitro challenge studies demonstrate that fermented fruit substrates fully suppress common foodborne pathogens, including *Escherichia coli*, *Salmonella enterica* Typhimurium, *Staphylococcus aureus*, and *Bacillus cereus*, within 24 to 48 hours of fermentation (Makras et al., 2006).

Table 2: Key Microorganisms Isolated from Pakistani Fruit and Botanical Fermentations and Their Functional Roles

Microorganism Species / Strain	Primary Isolation Source	Metabolic Pathway / Mechanism	Key Functional Outputs / Bioactive Enhancements	Probiotic & Safety Attributes
Lactiplantibacillus plantarum 022AE	Traditional Fermented Plant Substrates	Homofermentative Glycolysis, Tannase & Glucosidase activity	Lactic acid production, Exopolysaccharides, Plantaricin synthesis	Acid (pH 2.0) & Bile (0.3%) tolerant, High Caco-2 adhesion, No transferable AMR
Loigolactobacillus coryniformis BCH-4	Fermented Botanical Matrices	Heterofermentative Lactic Acid Fermentation	Organic acids, Bis-(2-ethylhexyl) phthalate, Cyclic dipeptides	Potent antifungal & aflatoxin-detoxifying activity, Anti-bacterial against gut pathogens
Saccharomyces cerevisiae	Fruit Fermentations	Anaerobic Alcoholic Fermentation (Glycolysis)	Ethanol, CO_2, Glycerol, Secondary aromatic esters	GRAS status, Stress tolerance,

	(Apricot, Grape)			Synergy with LAB in co-cultures
Acetobacter aceti	Traditional Vinegar (*Sirka*)	Aerobic Ethanol Oxidation ($ADH/ALDH$ enzymes)	Acetic acid, Acetoin, Organic acid complexes	Standard acetic acid producer, High acid tolerance, Pathogen suppression
Leuconostoc mesenteroides	Solar Fermentations (Kanji)	Heterofermentative Phosphoketolase Pathway	D-Lactic acid, Ethanol, CO_2, Dextran exopolysaccharides	Imparts characteristic effervescence, aroma, and rheological texture

4. Biochemical Mechanisms of Bioactive Compound Enhancement

The enhancement of bioactive potential during microbial fermentation is governed by specific enzymatic transformations that convert precursor molecules into biologically active metabolites. These transformations alter cell wall structures, modify polyphenolic profiles, and eliminate anti-nutritional compounds (Ali et al., 2026).

Cell Wall Hydrolysis and Phenolic Liberation

In unfermented fruits, a substantial fraction of polyphenols (up to 50%) is bound covalently to cell wall structural polymers via ester, ether, or acetal linkages with hemicellulose, pectin, and structural proteins. Humans lack the digestive enzymes necessary to cleave these complex linkages, resulting in poor upper-gastrointestinal absorption (Acosta-Estrada et al., 2014).

During fermentation, *Lactiplantibacillus plantarum* and associated microbes synthesize intracellular and extracellular enzymes, including β-glucosidase, pectinase, xylanase, and cellulose (Shahidi & Yeo, 2016). These enzymes hydrolyze the structural polysaccharide matrix of the fruit cell wall, releasing bound insoluble phenolics into the liquid juice phase. Fourier-Transform Infrared Spectroscopy (FTIR) analyses of fermented fruit beverages reveal structural breakdown in polysaccharide fingerprint regions ($900-1150\ \text{cm}^{-1}$) and enhanced hydrogen bonding and covalent cross-linking modifications, confirming structural cell wall degradation and compound liberation (Arnous & Meyer, 2010).

Biotransformation of Polyphenols, Flavonoids, and Ellagitannins

Once liberated, plant polyphenols undergo microbial transformation. Native fruit flavonoids occur predominantly as O-glycosides, where sugar moieties (glucose, rhamnose, galactose) increase water solubility but impair cellular membrane permeability (Alqudah & Claesen, 2024). Microbial β-glucosidases cleave these sugar moieties, converting flavonoid glycosides into free lipophilic aglycones (e.g., quercetin-3-O-glucoside to free quercetin). Aglycones exhibit significantly higher cellular uptake, greater membrane permeability, and enhanced free radical scavenging activity compared to their glycosylated parent molecules (Sallam et al., 2021).

In pomegranate (*Punica granatum*) and Jamun (*Syzygium cumini*) ferments, high-molecular-weight ellagitannins such as punicalagin undergo structural biotransformation. Microbial tannase (tannacyl hydrolase) cleaves the ester bonds of punicalagin, producing intermediate gallagyl esters and ultimately liberating free ellagic acid (Madeira et al., 2014). Ellagic acid is further metabolized by specific intestinal and fermentation microflora through lactone-ring cleavage and sequential dehydroxylation steps into urolithins (Urolithin A, Urolithin B). Urolithins possess potent systemic anti-inflammatory, anti-aging, and neuroprotective activities, crossing the blood-brain barrier to inhibit acetylcholinesterase ($AChE$) (Madeira Junior et al., 2015).

$$\text{Punicalagin (Complex Ellagitannin)} \xrightarrow{\text{Microbial Tannase}} \text{Ellagic Acid} \xrightarrow{\text{Dehydroxylases}} \text{Urolithin A / B}$$

In anthocyanin-rich substrates like black carrot Kanji and Phalsa ferments, fermentation preserves and transforms anthocyanins. While excessive heat degrades cyanidin glycosides, low-pH lactic fermentation stabilizes the flavylium cation form of anthocyanins. Microbial activity converts complex acylated anthocyanins into simpler anthocyanidins, increasing color intensity and maintaining long-term bio-accessibility (Pulido-Mateos et al., 2024).

Degradation of Anti-Nutritional Factors (ANFs)

Raw fruits, seeds, and botanicals contain anti-nutritional factors that interfere with nutrient absorption and metabolic processes. Prominent ANFs include phytates (phytic acid), oxalates, condensed tannins, alkaloids, and glucosinolates. Phytate forms insoluble chelates with essential divalent cations (Ca^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}), preventing their absorption in the human gut (Arsov et al., 2024).

Fermentation with *Lactiplantibacillus plantarum* drives marked enzymatic degradation of these ANFs through the synthesis of endogenous phytases and microbial decarboxylases. As documented in controlled plant fermentations:

- Phytate levels decrease significantly from 1.97 mg/100g to 0.43 mg/100g (over 78% reduction).
- Oxalate concentrations drop from 0.07 mg/100g to 0.02 mg/100g (Gbedo et al., 2025).
- Total alkaloid content reduces from 6.45 mg/100g down to 2.20 mg/100g.
- Glucosinolates degrade from 201 μmol/g to 10.50 μmol/g.

This degradation releases bound divalent minerals into free, bioavailable ionic forms while eliminating the bitter taste and gastrointestinal distress associated with raw botanical ANFs (Huang et al., 2019).

5. Functional Properties and Health Benefits

The biochemical transformations induced by microbial fermentation impart diverse health-promoting properties to Pakistani fruit-based functional foods. These benefits span distinct physiological domains, including enhanced antioxidant capacities, gastrointestinal probiotic efficacy, and targeted systemic health effects (Abuajah et al., 2015).

In Vitro and In Vivo Antioxidant Capacities

Fermentation enhances antioxidant capacity across multiple testing paradigms, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation neutralization, Ferric Reducing Antioxidant Power (FRAP), and oxygen radical absorbance capacity (ORAC) assays (Alam et al., 2013).

In comparative evaluations between unfermented juice controls and *Lactiplantibacillus plantarum*-fermented fruit matrices, fermented products exhibit increases of 40% to 120% in DPPH radical scavenging activity. This enhancement correlates directly with the accumulation of free gallic acid, ellagic acid, quercetin aglycones, and elevated Total Phenolic Content (TPC) (Shahidi & Samarasinghe, 2025).

The elevated radical scavenging efficiency protects cell membranes against lipid peroxidation by donating hydrogen atoms or electrons to reactive oxygen species (ROS) and reactive nitrogen species (RNS), neutralizing hydroxyl ($OH^{\cdot}$) and superoxide ($O_2^{\cdot -}$) radicals before they induce oxidative DNA damage (Fernandez-Panchon et al., 2008).

Cytotoxic, Anti-Inflammatory, and Neuroprotective Properties

Recent pharmacological screenings of Pakistani fermented fruit beverages reveal therapeutic activities against chronic, non-communicable diseases (Spagnuolo et al., 2018).

Antiproliferative and Cytotoxic Potential

Co-culture fermentations of Sea Buckthorn and monk fruit beverages using *Lactiplantibacillus plantarum* display selective cytotoxicity against HepG2 hepatocellular carcinoma cell lines. Cell viability assays indicate dose-dependent inhibition of cancer cell proliferation, driven by high concentrations of carotenoid esters, β-glucans, and solubilized flavonoids that induce intrinsic mitochondrial apoptosis pathways while sparing healthy fibroblast cell lines (HDFs) (Moacă et al., 2018).

Anti-Inflammatory and Hepatoprotective Effects
Pomegranate peel ferments and Phalsa extracts rich in ellagic acid and anthocyanins downregulate pro-inflammatory cytokines, specifically Tumor Necrosis Factor-alpha (TNF $-\alpha$), Interleukin-6 (IL -6), and Nuclear Factor kappa B (NF $-\kappa$B) signaling cascades. Animal model studies demonstrate that regular administration of fermented pomegranate preparations mitigates ferric nitrilotriacetate (Fe-NTA)-induced hepatotoxicity and carbon tetrachloride (CCl_4)-induced oxidative liver necrosis (Liu et al., 2015).

Neuroprotective and Anti-Cholinesterase Activity
Phenolic metabolites derived from pomegranate peel ferments (*Punica granatum*) act as dual inhibitors of acetylcholinesterase ($AChE$) and butyrylcholinesterase ($BChE$). Computational docking and in vitro enzyme inhibition assays confirm that punicalagin, ellagic acid, and gallic acid bind tightly to the catalytic triad of $AChE$, preventing acetylcholine breakdown in synaptic clefts. This neuroprotective mechanism offers potential dietary strategies for mitigating cognitive decline and supporting management of Alzheimer's disease (Wang & Tang, 2005).

6. Industrial Valorization, Regulatory Frameworks, and Quality Standards
Transitioning traditional Pakistani fermented foods from domestic artisanal production to commercial scale requires standardized bioprocessing, byproduct valorization, and compliance with national regulatory structures (Sun & Wang, 2026).

Industrial Scale-Up and Process Standardization
Traditional household fermentations rely on spontaneous microflora or back-slopping, leading to batch-to-batch variations in acidity, ethanol content, flavor profiles, and microbial safety. Standardizing commercial manufacture requires key technical implementations:

1. **Defined Starter Culture Inoculation**: Replacing spontaneous microflora with characterized, multi-strain starter cultures (e.g., *Lactiplantibacillus plantarum* 022AE combined with *Loigolactobacillus coryniformis* BCH-4) ensures predictable fermentation kinetics, rapid acidification, and consistent bioactive yields (Kockmann et al., 2011).

2. **Controlled Substrate Pre-Treatment**: Thermal processing or flash pasteurization of fresh fruit juices followed by controlled inoculation prevents wild yeast and mold spoilage.

3. **Optimized Fermentation Kinetics**: Maintaining bioreactor temperatures at $37°$C, agitation speeds at 120 rpm, and dissolved oxygen control maximizes cell density and metabolite production (Juyal et al., 2025).

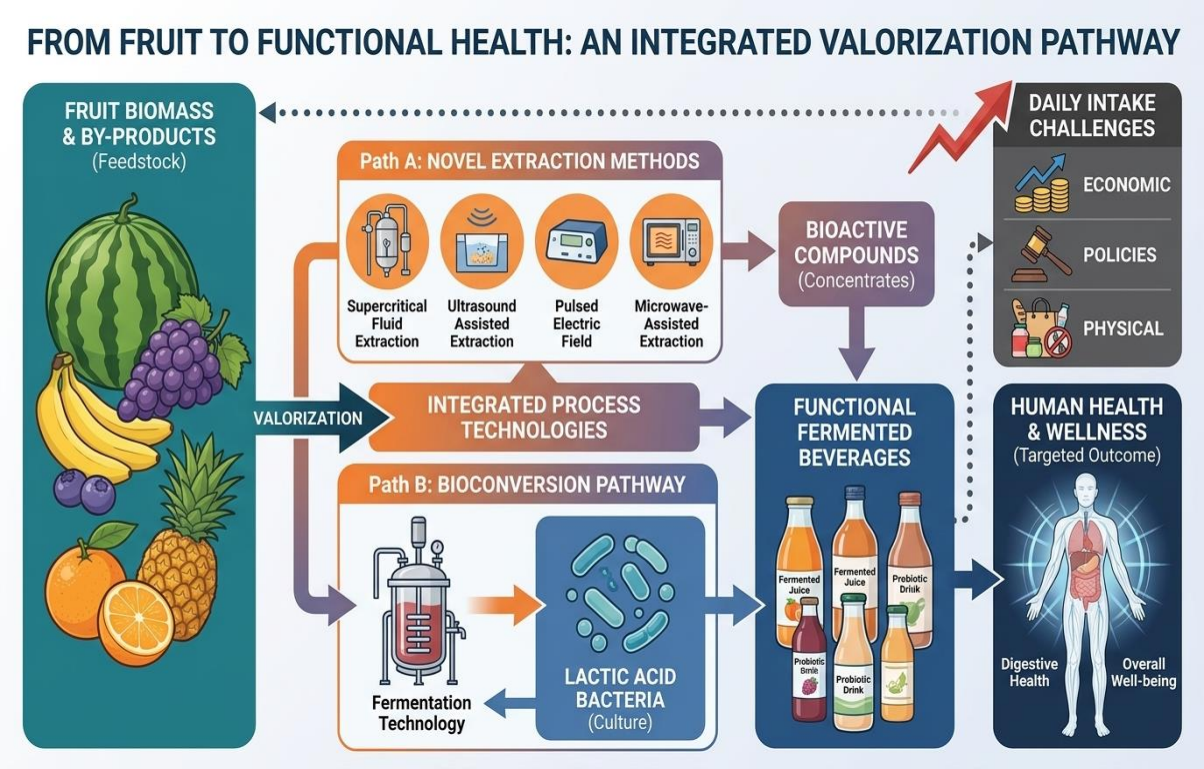


Figure 2: Integrated Valorization Pathway for Fruit Biomass and By-Products into Probiotic Functional Beverages and Targeted Health Outcomes

Solid-State Fermentation (SSF) and Pomace Valorization

Fruit processing operations across Pakistan generate substantial industrial waste, with fruit pomace (peel, pulp residues, and seeds) accounting for 40% to 50% of total fresh fruit weight. Disposing of this pomace creates environmental pollution and financial burdens. Solid-State Fermentation (SSF) presents an efficient valorization strategy to transform these low-cost byproducts into high-value functional additives (Šelo et al., 2021).

Industrial valorization can follow two parallel processing streams:

- Liquid juice fractions are directed toward controlled submerged fermentation in automated bioreactors using defined starter cultures to produce standardized probiotic beverages.
- Solid pomace fractions (peels, seeds, and pulp residues) undergo Solid-State Fermentation (SSF) under controlled moisture levels $(60 - 70\%)$ using *Lactiplantibacillus plantarum* or fungal strains (Siller-Sánchez et al., 2024).

Inoculating pomegranate peel, mango seed kernels, or citrus pomace under SSF conditions induces dense microbial growth. Microbial tannases and cellulases break down the dense lignocellulosic pomace matrix, releasing bound phenolics. Extracted ferments can be concentrated into bioactive powder extracts or utilized directly as high-antioxidant, low-ANF functional additives in livestock feeds and poultry rations, enhancing growth performance, gut morphology, and lipid oxidation stability in meat products (Filipe et al., 2020).

Regulatory Frameworks and Quality Standards in Pakistan

Commercial production of fermented fruit products in Pakistan is governed by federal and provincial regulatory bodies to ensure food safety, quality parameters, and trade compliance (Kemal, 2002).

Pakistan Standards and Quality Control Authority (PSQCA)

The PSQCA, operating under the Ministry of Science and Technology, formulates national quality standards. Compliance with specific Pakistan Standards (PS) is mandatory for commercial manufacture, packaging, and retail distribution:

- **PS: 4973-2009 (Fruit Juices & Nectars)**: Establishes composition, minimum Brix levels, permitted food additives, and strict microbiological tolerances for unfermented and fermentable fruit beverages (Mirza & Shafi, 2026).
- **PS: 1738-2009 (Orange Juice) & PS: 1739-2009 (Apple Juice)**: Specify mandatory chemical and physical quality criteria, including a minimum titratable acidity of 0.5% w/v (calculated as anhydrous citric acid).
- **PS: 3602-2018 (Synthetic Vinegar) & PS: 523 (Fruit Vinegar)**: Define specifications for acetous ferments. PS: 3602-2018 mandates that vinegar labeling clearly differentiate between synthetic acetic acid dilutions ($4 - 5\%$ m/v) and naturally fermented fruit vinegars (*Fruit Sirka*), prohibiting deceptive labeling (Sultan et al., 2025).
- **PS: 520-2013 (Pickles)**: Governs oil-, brine-, and acid-preserved fruits and botanicals, defining acceptable salt concentrations, titratable acidity, and heavy metal limits (e.g., Lead ≤ 0.2 mg/kg, Arsenic ≤ 0.1 mg/kg).

Provincial Food Authorities

The Punjab Food Authority (PFA), Khyber Pakhtunkhwa Food Safety Authority (KPFSA), Sindh Food Authority (SFA), and Balochistan Food Authority (BFA) enforce PSQCA standards within their respective provincial jurisdictions (Khan et al., 2020). Under Council of Common Interests (CCI) decisions, provincial authorities adopt national PSQCA quality and labeling standards while executing licensing, factory inspections, market surveillance, and microbiological testing 9 Rabbani & Abbasi, 2017).

Commercial manufacturers of fermented fruit products must meet stringent microbiological thresholds prior to distribution:

- Total Plate Count (TPC): < 1000 CFU/g (for non-probiotic finished products).
- Coliforms: < 10 CFU/g.
- Yeasts and Molds: < 100 CFU/g.
- Pathogens (*E. coli*, *Listeria monocytogenes*, *Salmonella*): Completely absent in 25 g samples (Khan & Haider, 2003).

For designated probiotic functional beverages, regulatory standards require clear labeling of specific strains, verified viable cell counts exceeding 10^7 CFU/mL throughout shelf life, and proof of non-transferable antibiotic resistance profiles (Irshad et al., 2021).

Table 3: Relevant Pakistan Standards (PSQCA) and Regulatory Parameters for Fermented and Fruit-Based Products

Standard Code (PS Number)	Regulatory Product Category	Mandatory Quality & Chemical Parameters	Microbiological Tolerances & Safety Limits	Industrial Relevance & Application
PS: 4973-2009	Fruit Juices and Nectars	Minimum Brix levels per fruit type, Authenticity criteria, Permitted GMP additives	$TPC <$ 1000 CFU/g, Coliforms $<$ 10 CFU/g, Molds $<$ 100 CFU/g	Baseline quality standard for commercial fermentable fruit juice bases
PS: 3602-2018	Synthetic & Fermented Vinegar	Acetic acid strength ($4.0 - 5.0\%$ w/v), Heavy metal limits (Lead ≤ 0.2 mg/kg)	Absence of pathogenic contamination, Compliance with Halal PS: 3733	Differentiates synthetic vinegar from fermented fruit vinegars (*Sirka*)

PS: 520-2013	Pickles (Fruit & Botanical in Brine/Oil)	Mandatory titratable acidity, Minimum salt/oil cover, Moisture control	Absence of *E. coli*, *Listeria*, and pathogenic spore-formers	Governs solid/semi-solid fruit ferments like Anardana & pickled mango/carrots
PS: 1738-2009 / PS: 1739-2009	Specific Fruit Juices (Orange / Apple)	Titratable acidity ≥ 0.5% w/v (as citric acid), Minimum Brix (11.2 – 11.8)	Coliforms < 10 CFU/g, Pathogens absent per 25g	Governs fruit substrate integrity before probiotic strain inoculation
PS: 527-2016	Concentrated Fruit Juices	Reconstitution Brix verification, Soluble solids standards	Strict hygienic packaging, Free of adulterants	Standards for commercial fruit concentrates used in industrial ferments

Conclusion

This review demonstrates that traditional fruit-based fermented foods of Pakistan constitute a valuable but underutilized resource of functional nutrition and therapeutic bioactives. The microbial consortia indigenous to these ferments particularly *Lactiplantibacillus plantarum*, *Loigolactobacillus coryniformis*, and *Saccharomyces cerevisiae* drive sophisticated biochemical transformations that enhance the bioavailability and bioactivity of native plant compounds. Key mechanisms include enzymatic cell wall hydrolysis that liberates bound phenolics, glycosidase-mediated conversion of flavonoid glycosides to lipophilic aglycones, tannase-catalyzed hydrolysis of ellagitannins into ellagic acid and urolithin precursors, and phytase-driven degradation of anti-nutritional factors. These transformations collectively elevate antioxidant capacity, generate probiotic metabolites, and impart clinically relevant properties including anti-inflammatory, hepatoprotective, neuroprotective, hypoglycemic, and cytotoxic activities against cancer cell lines. However, significant gaps remain between traditional artisanal production and commercial standardization. Batch-to-batch variability, reliance on spontaneous microflora, and limited process control constrain scalability and safety assurance. Future research should prioritize the development of defined multi-strain starter cultures tailored to specific fruit substrates, optimization of fermentation parameters in controlled bioreactor systems, and clinical validation of health claims through randomized controlled trials. Furthermore, industrial valorization strategies particularly solid-state fermentation of fruit pomace for bioactive extraction and livestock feed supplementation offer sustainable pathways for waste reduction and economic value creation. Regulatory harmonization under PSQCA standards, with clear labeling requirements for probiotic strains and viable cell counts, will be essential for consumer safety and market acceptance. By integrating traditional knowledge with modern biotechnological approaches, Pakistan can develop a robust functional food industry that honors its culinary heritage while addressing contemporary nutritional and public health challenges.

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