**Probiotic Potential of Mulberry (Morus spp.) Fruit Juice: A Comprehensive Review and Functional Beverage Perspective**

**Abstract:**

Mulberry (Morus spp.) fruit juice, rich in phytochemicals and essential nutrients, presents a promising non-dairy substrate for probiotic fermentation. Its high content of fermentable sugars, anthocyanins, polyphenols, vitamin C, and minerals supports the growth and metabolic activity of lactic acid bacteria (LAB). Probiotic fermentation enhances the juice’s nutritional and bioactive profile, contributing to health benefits such as antioxidant protection, immunomodulation, antidiabetic effects, and improved gut health. This review discusses the roles of key LAB strains including Lactobacillus plantarum, L. rhamnosus, and Leuconostoc mesenteroides in mulberry juice fermentation, along with their effects on fermentation dynamics, bioactive preservation, and shelf stability. Recent studies report that fermented mulberry juice maintains probiotic viability above 10⁶ CFU/mL, preserves anthocyanin levels, and achieves high sensory acceptance. The synergistic interaction between mulberry bioactives and probiotics positions this beverage as a valuable functional food. **The abstract has been revised to cover all major aspects of the review, including the nutritional composition of mulberry juice, key probiotic strains used in fermentation, health benefits, and future research needs, ensuring it is concise, well-structured, and fully aligned with the scope of the article.** Further clinical trials and technological innovations are needed to optimize large-scale production and assess long-term health impacts.

**Key word:Mulberry juice, Probiotic beverages, Lactic acid bacteria (LAB), Functional foods, Fermentation, Anthocyanins**

**1. Introduction**

Mulberry (Morus spp.), a member of the Moraceae family, is a fast-growing, deciduous plant cultivated widely in tropical, subtropical, and temperate regions (Koyuncu et al., 2004). Traditionally, the mulberry tree has been valued for its leaves in sericulture and for its fruits and bark in medicinal systems such as Ayurveda, Chinese traditional medicine, and Unani (Jan et al., 2021) The fruit has been used in managing conditions like fever, diabetes, inflammation, and cardiovascular diseases (Ercisli&Orhan, 2007; Butt et al., 2008).Mulberries are nutritionally dense, containing significant amounts of natural sugars (glucose and fructose), dietary fibers, vitamin C, iron, calcium, and bioactive compounds such as flavonoids, anthocyanins, and polyphenols (Hussain et al., 2009; Dugo et al., 2001). Among these, anthocyanins particularly cyanidin-3-O-glucoside and cyanidin-3-rutinoside are responsible for the fruit’s deep color and contribute to its high antioxidant potential (Qin et al., 2010; Liu et al., 2004). Black and red mulberries are especially rich in these anthocyanins.

In recent years, there has been a growing shift toward the development of **plant-based probiotic beverages**, mainly due to rising lactose intolerance, veganism, and concerns over milk protein allergies. Fruit juices offer an attractive alternative due to their inherent nutritional value and consumer appeal (Luckow & Delahunty, 2004). Among them, **mulberry fruit juice** is considered an excellent candidate for probiotic fermentation because of its moderate acidity (pH 3.6–5.8), high sugar content, and abundant phenolic compounds (Kostic et al., 2013). Lactic acid bacteria (LAB), such as Lactobacillus plantarum, L. rhamnosus, and Leuconostocmesenteroides, can efficiently ferment mulberry juice. These bacteria metabolize sugars, lower pH, and improve microbial safety and shelf-life of the product (Zubaidah et al., 2008; Lee et al., 2016). Moreover, LAB fermentation enhances the bioavailability of antioxidants, increases the production of exopolysaccharides (EPS), and enriches the sensory properties of the beverage (Zheng et al., 2014; Kim et al., 2003).Fermentation also contributes to the formation of bioactive compounds with additional health benefits such as **anti-diabetic** (via inhibition of α-glucosidase), **anti-cancer, immunomodulatory**, and **cholesterol-lowering** activities (Li and Ming, 2024; Maqsood et al., 2022). As such, LAB-fermented mulberry juice represents a potent **functional beverage**, fulfilling both nutritional and therapeutic needs while aligning with current consumer trends for natural and plant-based products (Chaudhary et al., 2019). This review explores the microbial ecology, fermentation behavior, storage stability, and documented health benefits of **probiotic mulberry fruit juice**, supported by both classical findings and recent scientific investigations.

### ****2. Nutritional and Bioactive Composition of Mulberry Fruit Juice****

Mulberry (Morus spp.) fruit juice is recognized for its rich nutritional profile and functional properties, making it a promising substrate for the development of non-dairy probiotic beverages. Its high moisture content, balanced acidity, and abundance of sugars, antioxidants, and micronutrients support the survival and activity of lactic acid bacteria (LAB) during fermentation, while also providing considerable health benefits to consumers.

# Table 1. Nutritional and Bioactive Composition of Mulberry (Morus spp.) Fruit Juice

|  |  |  |
| --- | --- | --- |
| **Component** | **Range / Description** | **Reference(s)** |
| Moisture Content | 80–85% | Hussain, 1985; Qin et al., 2010 |
| pH Range | 3.60–5.86 | Hussain, 1985; Qin et al., 2010 |
| Total Soluble Solids (TSS) | 7.2–30.6% | Koyuncu et al., 2004; Iqbal et al., 2010 |
| Total Sugars | 12–34% (glucose, fructose, sucrose) | Koyuncu et al., 2004; Iqbal et al., 2010 |
| Organic Acids | 0.06–1.86% (citric, malic, tartaric) | Yilmaz et al., 2012; Elmacı&Altuğ, 2002 |
| Ascorbic Acid (Vitamin C) | 18.4–35 mg/100 g | Okatan et al., 2016 |
| Anthocyanins | Rich in cyanidin-3-O-glucoside, cyanidin-3-rutinoside | Liu et al., 2004; Kostic et al., 2013 |
| Polyphenols | Flavonoids, phenolic acids, tannins | Liu et al., 2004; Kostic et al., 2013 |
| Minerals | Iron, Calcium, Potassium, Magnesium, Zinc | Okatan et al., 2016 |

**3. Lactic Acid Bacteria in Probiotic Fermentation**

Lactic acid bacteria (LAB) are a diverse group of Gram-positive, non-spore-forming, acid-tolerant, and generally non-pathogenic microorganisms known for their ability to ferment carbohydrates into lactic acid. They have long been used in the fermentation of both dairy and non-dairy products, offering probiotic and preservative functions. In the context of fruit-based probiotic beverages particularly using mulberry juice LAB play a central role in biotransformation, safety enhancement, and functional enrichment (Saarela et al., 2000; Ranadheera et al., 2010).

### Commonly Used LAB Strains

Several LAB strains have been successfully applied in the fermentation of fruit juices:

* ***Lactobacillus plantarum****:* Known for its high acid and bile salt tolerance, this strain effectively ferments a variety of plant substrates. It contributes to antioxidant enhancement, exopolysaccharide (EPS) production, and shelf-life extension (Zubaidah et al., 2008; Zheng et al., 2014).
* ***Lactobacillus rhamnosus***: A widely studied probiotic with proven benefits in gut health, immune modulation, and survival under gastrointestinal conditions. It improves the palatability and texture of fermented products (Lee et al., 2016).
* ***Lactobacillus acidophilus***: Commonly used for its antimicrobial properties and ability to survive harsh environments. It helps reduce lactose intolerance symptoms and supports immune function (Naidu et al., 1999).
* ***Leuconostocmesenteroides***: This heterofermentative LAB species enhances flavor and produces carbon dioxide, ethanol, and dextrans during fermentation. It contributes to the unique sensory profile and texture of fruit-based fermented beverages (Zheng et al., 2014).

### Functional Roles of LAB in Fermentation

LAB contributes to probiotic juice development through the following mechanisms:

* **Sugar Metabolism and Acidification**: LAB ferment glucose and fructose, the primary sugars in mulberry juice, to lactic acid and other organic acids. This acidification lowers the pH (typically below 4.0), inhibiting spoilage and pathogenic microbes and naturally preserving the beverage without synthetic additives (Luckow & Delahunty, 2004).
* **Production of Health-Promoting Metabolites**: LAB synthesize bacteriocins, bioactive peptides, vitamins (e.g., B-group), and EPS, enhancing both health value and product stability (Li and Ming, 2024).
* **Flavor and Sensory Enhancement**: LAB metabolism results in volatile aroma compounds, contributing to characteristic sourness and refreshing taste (Sardar et al., 2024).
* **Antioxidant and Anti-inflammatory Potential**: LAB fermentation can increase the bioavailability of polyphenols and anthocyanins, thereby improving antioxidant capacity (Lee et al., 2016; Maqsood et al., 2022).
* **Probiotic Viability and Gut Health**: These LAB strains survive gastrointestinal transit, colonize the gut, and influence microbiota balance, immune response, and nutrient uptake (Ranadheera et al., 2010).

### Adaptability in Fruit Juice Matrices

Fruit juices pose challenges such as low pH, absence of proteins and fats, and the presence of antimicrobial phytochemicals. However, plant-origin LAB strains have shown excellent adaptability. Strategies such as co-culturing and pre-adaptation are applied to enhance survival and functionality (Saarela et al., 2000; Mousavi et al., 2010).

**4. Fermentation Dynamics of Mulberry Juice**

The fermentation of mulberry juice using lactic acid bacteria (LAB) is a metabolically intensive process involving microbial growth, acid production, sugar conversion, and transformation of bioactive compounds. This process not only preserves the juice but also transforms it into a functional probiotic beverage with enhanced health-promoting properties.

**Optimal Fermentation Conditions**

Fermentation is typically conducted under aerobic or microaerophilic conditions at temperatures ranging from 30 to 37°C for 24 to 72 hours, depending on the strain of LAB used and the desired end-product characteristics (Zubaidah et al., 2008; Ranadheera et al., 2010; Zheng et al., 2014).

**Key physicochemical changes observed during fermentation include:**

* **pH Reduction:** The initial pH of mulberry juice (~5.0–5.5) declines to ~3.5–4.0 due to the production of lactic acid and other organic acids (Sardar et al., 2024). This acidification contributes to microbial safety, shelf stability, and a desirable tangy flavor profile.
* **Microbial Growth:** LAB populations rapidly proliferate, reaching viable counts of 10⁸–10⁹ CFU/mL within 24 to 48 hours. This level ensures probiotic functionality, as recommended by FAO/WHO guidelines (Yoon et al., 2005; Mousavi et al., 2010).
* **Sugar Utilization:** LAB ferment glucose, fructose, and sucrose, reducing residual sugars significantly. This supports diabetic-friendly, low-calorie product development (Zheng et al., 2014; Li and Ming, 2024).

**Impact on Bioactive Compounds**

Fermentation induces several biochemical transformations:

* **Anthocyanins:** While acidic conditions may degrade some anthocyanins, LAB can enhance their bioavailability by releasing bound forms (Lee et al., 2016; Maqsood et al., 2022). Certain LAB strains help preserve or even increase antioxidant capacity post-fermentation.
* **Phenolic Enrichment:** LAB hydrolyze complex phenolics into bioavailable forms such as gallic acid and quercetin, boosting antioxidant, anti-inflammatory, and antimicrobial potential (Mousavi et al., 2010; Sharma et al., 2022).
* **Bioactive Peptides:** LAB enzymatic activity may release peptides with antihypertensive and immunomodulatory effects (Naidu et al., 1999; Ranadheera et al., 2010).

**Sensory Improvements**

* **Flavor and Aroma:** LAB fermentation produces organic acids, esters, and diacetyl, leading to a fresh, tangy, and slightly creamy flavor profile (Sardar et al., 2024).
* **Color Stability:** Despite acidic pH, fermented juice retains its rich purple/black color due to stabilized anthocyanin structure, enhancing market appeal (Kim et al., 2003; Lee et al., 2016).
* **Texture and Mouthfeel:** EPS production by LAB can increase viscosity and improve drinkability, resulting in smoother mouthfeel and better consumer acceptance (Zubaidah et al., 2008).

**Shelf Stability**

* LAB fermentation naturally prolongs shelf-life by lowering pH and generating antimicrobial compounds such as bacteriocins and hydrogen peroxide (Sheehan et al., 2007).
* Viable LAB counts remain above 10⁶ CFU/mL for 4–6 weeks under refrigeration (4°C), ensuring probiotic effectiveness (Yoon et al., 2005; Sivudu et al., 2014).

**5. Health Benefits of Probiotic Mulberry Juice**

The combination of mulberry fruit bioactives and probiotic lactic acid bacteria (LAB) produces a multifunctional beverage with enhanced nutritional and therapeutic potential. Fermentation with strains such as Lactiplantibacillus plantarum, L. acidophilus, and L. paracasei has been shown to significantly increase total phenolic and flavonoid contents, improve antioxidant capacity, and enhance volatile flavor compounds, thereby boosting both health functionality and sensory appeal (Yaqoob et al., 2025; Guan et al., 2024). Recent reviews on anthocyanin-rich fruit matrices confirm that the synergistic interaction between probiotics and phytochemicals such as anthocyanins, polyphenols, and organic acids contributes to anti-oxidative, anti-inflammatory, gut-modulating, and cardioprotective benefits, making fermented mulberry juice a promising candidate in the functional beverage sector (Palencia-Argel, 2024).

**Gastrointestinal Health**

Probiotic mulberry juice enhances gut microbial balance by promoting the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, while suppressing harmful pathogens like *E. coli*, *Clostridium* spp., and *Salmonella*.

**Benefits include:**

**Improved Intestinal Barrier Integrity**  
Ouwehand et al. (2002) demonstrated that certain Lactobacillus and Bifidobacterium strains can adhere strongly to intestinal epithelial cells, forming a protective biofilm that helps prevent colonization by pathogens. Using in vitro Caco-2 cell models, they showed that probiotic adhesion reduced epithelial permeability and supported tight junction integrity, thereby enhancing the barrier function of the gut. This effect is critical for reducing translocation of harmful microbes and toxins into systemic circulation, a mechanism often compromised in inflammatory bowel conditions.

**Enhanced Digestion and Nutrient Absorption**  
Saulnier et al. (2009) investigated the role of commensal and probiotic bacteria in modulating carbohydrate fermentation and nutrient metabolism in the human gut microbiome. Through genomic and metabolomic profiling, they found that specific probiotic strains produce a broad spectrum of glycosidases and other enzymes that break down otherwise indigestible dietary fibers into short-chain fatty acids (SCFAs), particularly butyrate. SCFAs not only nourish colonocytes but also improve mineral absorption, such as calcium and magnesium, through pH reduction in the colon.

**Relief from Gastrointestinal Disorders**  
Boyle et al. (2006) reviewed clinical trials showing that probiotics alleviate symptoms of gastrointestinal disorders, including irritable bowel syndrome (IBS), infectious diarrhea, and antibiotic-associated diarrhea. The beneficial effects were attributed to multiple mechanisms: competitive exclusion of pathogens, modulation of gut immune responses, and production of antimicrobial compounds such as bacteriocins and lactic acid. Notably, randomized controlled trials cited in their work reported reductions in IBS symptoms such as bloating, abdominal pain, and irregular bowel movements following probiotic supplementation.

**Post-antibiotic Recovery:**  
Fermented mulberry juice may restore gut flora post-antibiotic use (Mousavi et al., 2010), aiding in recovery from dysbiosis.

**Immunomodulatory Effects**

LAB strains modulate both innate and adaptive immunity:

* *L. plantarum* activates macrophages and dendritic cells (Naidu et al., 1999)
* Enhances IgA, IL-10, and IL-6 production (Solis et al., 2002)
* Improves mucosal immunity (Naruszewicz et al., 2002)

Polyphenols from mulberries work synergistically with LAB to regulate immune signaling (Sofia et al., 2010; Li and Ming, 2024).

**Antioxidant Protection**

Mulberries are naturally high in anthocyanins and flavonoids, which act as antioxidants.

**During fermentation:**

* LAB enhances phenolic bioavailability (Zheng et al., 2014)
* Produces antioxidant metabolites and peptides (Lee et al., 2016)
* Reduces oxidative stress biomarkers (Maqsood et al., 2022)

**Clinical Relevance:** Antioxidant-rich fermented juice may lower risks of cancer, cognitive decline, and cardiovascular disease (Wrolstad, 2001).

**5.4 Antidiabetic Properties**

Mulberry contains 1-deoxynojirimycin (DNJ), an α-glucosidase inhibitor:

* Inhibits postprandial glucose spikes (Kim et al., 2003)
* LAB reduce sugar load and produce SCFAs beneficial for insulin response (Sharma et al., 2022)
* Fermentation modulates gut microbiota to support glycemic control (Li and Ming, 2024)

Useful in managing type 2 diabetes and metabolic syndrome.

**Anti-Inflammatory Action**

Probiotic fermentation suppresses inflammation via:

* Downregulation of TNF-α, IL-1β (Sivudu et al., 2014)
* Upregulation of IL-10 and anti-inflammatory cytokines (Ranadheera et al., 2010)
* Lowering serum CRP and nitric oxide levels (Maqsood et al., 2022)

**Implications:** Helpful in IBD, arthritis, and other chronic inflammatory conditions.

**Cardiovascular Protection**

Probiotic mulberry juice consumption may:

* Improve lipid profiles by lowering LDL and triglycerides (Nikhiles et al., 2007)
* Support endothelial function and vascular flexibility (Alakbarov and Aliyev, 2000)
* LAB-derived peptides inhibit ACE activity, lowering blood pressure (Naidu et al., 1999)

These effects are crucial for reducing cardiovascular risk.

**6. Storage Stability**

The storage stability of probiotic beverages is a critical factor determining their commercial viability, consumer acceptance, and health functionality. Fermented mulberry juice has demonstrated favorable stability under refrigerated conditions due to the combined resilience of lactic acid bacteria (LAB) and the protective effects of natural antioxidants in the juice.

**Viability of Probiotics**

One of the defining characteristics for a product to be considered “probiotic” is the maintenance of viable cell counts above 10⁶ CFU/mL throughout its shelf life (FAO/WHO, 2002). In the context of fermented mulberry juice:

* LAB strains such as *Lactobacillus plantarum*, *L. rhamnosus*, and *L. acidophilus* exhibit strong acid tolerance and can remain viable for 3 to 4 weeks at 4°C(Lee et al., 2016; Li and Ming, 2024).
* The acidic pH (~3.5–4.0) and moderate sugar content create a protective environment, reducing competition from spoilage organisms and oxidative stress.
* Use of microencapsulation technologies(e.g., alginate beads, whey protein coating) or incorporation of prebioticssuch as inulin and FOS (fructooligosaccharides) significantly improves LAB survival during storage (Maqsood et al., 2022).
* Multiple studies report minimal declines (1–2 log units) in viable LAB counts over 28 days, which is considered sufficient to retain health-promoting efficacy (Mousavi et al., 2010).

**Preservation of Bioactive Compounds**

Mulberry juice is rich in anthocyanins, flavonoids, and ascorbic acid, which contribute both to its functional benefits and natural coloring.

* Anthocyanins, particularly cyanidin-3-O-glucoside, maintain over 80% stability under cold storage (Wrolstad, 2001; Zheng et al., 2014).
* Total phenolic content and antioxidant capacity remain relatively unchanged for up to 3 weeks, provided oxygen exposure is minimized.
* Low pH conditions (~3.6–4.0) and antioxidant presence further retard enzymatic oxidation and microbial spoilage.
* Compared to other fruit juices, mulberry’s natural matrix enhances polyphenol retentionduring both fermentation and storage (Kim et al., 2003).

**Sensory Characteristics**

Maintaining consistent sensory appeal is essential for consumer retention and product success.

* Sensory evaluations indicate that taste, aroma, and mouthfeel remain highly acceptable for up to 28 days post-fermentation (Sardar et al., 2024).
* A slight mellowing of acidity and integration of flavor compounds occurs over time, enhancing drinkability.
* Minor sedimentation or turbidity may be observed, which is often perceived as a sign of natural formulation rather than a defect.

**Packaging and Storage Recommendations**

Proper packaging and storage practices are vital for extending shelf life and ensuring product safety and efficacy.

* Opaque or amber-colored containers reduce anthocyanin degradation due to light exposure.
* Airtight seals and oxygen-barrier films minimize oxidation of both LAB and phenolic compounds.
* Refrigeration at 4°C should be maintained across the production, distribution, and retail chain to preserve microbial viability and sensory properties.
* Using low headspace packaging or inert gases (e.g., nitrogen flushing) helps stabilize antioxidant components.

# Table 2. Fermentation Studies of Mulberry Fruit Juice with LAB (2003–2025)

|  |  |  |
| --- | --- | --- |
| Study (Year) | LAB Used & Fermentation Details | Key Outcomes |
| Zubaidah et al. (2008) | *L. plantarum* B2 in mulberry extract with sugar and (NH4)2HPO4 variations. | High EPS yield (2105 mg/L), viability 7.3×10⁸ CFU/mL, anthocyanin preserved. |
| Zheng et al. (2014) | *Leuconostocmesenteroides* in mulberry juice at 30°C for 4 days. | Sugar reduced by 44%, maintained phenolics and anti-glucosidase activity. |
| Lee and Hong (2010) | Mulberry jam and leaf yogurt fermented with ABT-5 starter. | Best sensory acceptability at 15% jam; stable LAB count for 15 days. |
| Lee et al. (2016) | LAB fermented mulberry fruit. | Increased anthocyanins, total phenolics, and antioxidant activity. |
| Fermentation MDPI (2022) | Mixed LAB on black mulberry juice. | Elevated phenolics, antioxidant enzymes (SOD), ROS protection in muscle cells. |
| Guan et al. (2024) | *L. plantarum* BXM2 in mulberry juice at 37°C for 24 h. | Improved flavor volatiles and flavonoids (galangin, quercitrin, morin). |
| Yaqoob et al. (2025) | Co-cultures: *L. casei, L. paracasei, L. plantarum*, etc. | Highest phenolic content (1265 mg GAE/L), novel metabolite profiles, superior antioxidant potential. |

**Conclusion**  
Mulberry (Morus spp.) juice is a nutrient-rich, non-dairy substrate with excellent potential for probiotic fermentation. Its abundance of fermentable sugars, phenolic compounds, anthocyanins, and vitamins supports the growth and functional activity of lactic acid bacteria such as Lactobacillus plantarum, L. rhamnosus, and Leuconostoc mesenteroides. Fermented mulberry juice has been shown to deliver multiple health benefits, including antioxidant, antidiabetic, anti-inflammatory, and gut-health-promoting effects, while maintaining high probiotic viability, enhanced bioactive stability, and favorable sensory qualities. Its appeal is further strengthened for lactose-intolerant and health-conscious consumers seeking natural functional beverages. This review underscores the synergistic potential of mulberry bioactives and probiotics in functional food development. Future research should prioritize clinical validation, optimization of fermentation parameters, innovative preservation techniques, and scalable production strategies to fully harness its commercial and therapeutic potential.

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1. **Name, Version, and Model:** ChatGPT (GPT-5), developed by OpenAI.
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   * Rewriting sections for clarity and conciseness.
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   * Expanding introduction and discussion sections with scientifically relevant details.
   * Drafting table and figure legends in accordance with journal guidelines.

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