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Review Paper

Review On Formulation and Evaluation of Beetroot Juice as Prebiotics

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ABSTRACT

Beetroot juice has gained significant attention for its health-promoting properties, including its potential as a functional food supporting gut health. Rich in bioactive compounds such as nitrates and polyphenols, beetroot juice exhibits antioxidant, anti-inflammatory, and vasodilatory effects. Recent studies suggest that beetroot juice may also act as a prebiotic, promoting the growth of beneficial gut microbiota, particularly those involved in nitrate metabolism and short-chain fatty acid production.

INTRODUCTION

With improvements in economic status, there has been a growing interest in health and well-being, leading to an increased consumption of natural and health-promoting foods. As a result, the food industry is facing challenges in developing value-added, health-enhancing products and sourcing new, phytochemical-rich raw materials. Health professionals are encouraging consumers to benefit from probiotic foods for better health. Currently, dairy products dominate the probiotic food market, but they have limitations due to milk allergies and cholesterol content. Non-dairy alternatives can substitute dairy-based probiotics and offer additional health benefits. The development of probiotic food formulations is also

advancing the creation of dietary supplements, nutraceuticals, and prebiotics. Fruits and vegetables are highly valued for their carbohydrate, mineral, vitamin, dietary fiber, and antioxidant content, which support body repair, maintenance, and the building of alkaline reserves. Furthermore, they are free from dairy allergens. India, being a leading producer of fruits and vegetables, faces significant challenges with spoilage due to improper processing and poor postharvest management. Nevertheless, fruits and vegetables are naturally healthy, refreshing, and rich in beneficial nutrients, making them ideal substrates for non-dairy probiotics. Fermentation of these foods can improve their nutritional value, digestibility, shelf life, safety, and sensory

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qualities, while preserving nutrients that would otherwise be lost during thermal food processing. The growing interest in functional foods has brought attention to beetroot juice as a potential modulator of gut health. Traditionally valued for its cardiovascular and anti-inflammatory benefits, beetroot juice is rich in bioactive compounds, including nitrates, betalains, polyphenols, and dietary fiber, which contribute to its broad range of health-promoting properties. Recent research suggests that beetroot juice may also influence gut microbiota composition, acting as a prebiotic to support the growth and activity of beneficial bacteria. The gut microbiome plays a pivotal role in maintaining overall health, influencing processes such as digestion, immune regulation, and metabolic function. Probiotics, live microorganisms that confer health benefits when consumed in adequate amounts, have been widely studied for their ability to restore gut microbiota balance. However, the efficacy of probiotics can be enhanced by prebiotic substances, which provide a nutrient source for beneficial bacteria. Beetroot juice, with its unique profile of bioactive compounds, shows promise in serving as a natural prebiotic that complements and enhances the effects of probiotics. This review explores the role of beetroot juice in modulating gut microbiota, its interactions with probiotic strains, and its potential as a functional food for improving gut health. It also addresses the current challenges in this field and outlines directions for future research to better understand.

the mechanisms and applications of beetroot juice in probiotic therapy.

■ **Literature Review:**

- Panghal et al. (2017) The literature review for the study "Development of Prebiotic Beetroot Drink) primarily focuses on the potential of beetroot juice as a substrate for probiotic cultures and its health benefits. Beetroot's Nutritional Profile*: Beetroot is rich in

essential nutrients such as vitamins, minerals, antioxidants, and dietary nitrates. These attributes contribute to its health-promoting properties, including cardiovascular benefits and antioxidant effects. Probiotic offer dual benefits of probiotics and the functional properties of the base material (in this case, beetroot). Such drinks can improve gut health, strengthen immunity, and provide enhanced bioavailability of nutrients. The research emphasizes the growing interest in using probiotic fermentation to enhance the nutritional and functional properties of food. Fermentation with lactic acid bacteria can improve digestibility, increase antioxidant content, and provide a natural preservation method. The study by Kushwaha et al. (2017) explores an eco-friendly approach to extract betalains and other phytochemicals from beetroot pomace, a byproduct of juice production. This research aligns with the global emphasis on waste valorization, where agricultural byproducts are repurposed for high-value applications. Beetroot pomace, often discarded as waste, was demonstrated to be a rich source of bioactive compounds, particularly betalains, which have antioxidant, anti-inflammatory, and anticancer properties. The extracted betalains can be used as natural food colorants and functional ingredients in nutraceuticals and cosmetics. This promotes the circular economy by converting waste into valuable resources. The study provides a framework for scaling up eco-friendly extraction processes, enabling industries to adopt sustainable practices without compromising efficiency. Stability issues with betalains under varying pH and temperature conditions were noted, necessitating further research into preservation techniques during storage and application. The study systematically optimized factors such as



solvent concentration, temperature, pH, and extraction time to maximize the yield of betalains and phytochemicals. This research highlights the potential of beetroot pomace as a sustainable source of high-value phytochemicals. By focusing on eco-friendly extraction methods, it contributes to reducing waste and advancing sustainable practices in the food, pharmaceutical, and cosmetic industries. This study contributes to the broader field of waste and biomass valorization by demonstrating a practical method for converting agro-industrial waste into valuable products, aligning with the goals of circular economy and sustainable development. Attia et al. (2013) focus on the characterization of red pigments extracted from red beet (*Beta vulgaris* L.) and their potential applications as antioxidants and natural food colorants. The study examines the chemical composition, stability, and bioactivity of betalains, the primary red pigments in beets, to assess their suitability for use in various industries, especially in food and pharmaceuticals. The authors explore the antioxidant properties of betalains, which are known to combat oxidative stress and have health-promoting effects. These properties make betalains a valuable alternative to synthetic antioxidants and preservatives. In addition to antioxidant activity, betalains are also evaluated for their color stability, which is crucial for their use in food products, as color degradation during processing and storage can limit their commercial viability. The study provides a detailed analysis of the betalain profile, including betacyanins (the red pigments) and betaxanthins, which contribute to the overall bioactivity and color. Besides antioxidant activity, the study discusses other potential health benefits, such as anti-inflammatory and anticancer properties, which

further enhance the value of beetroot pigments in nutraceutical applications. The stability and vibrant red color of betalains position them as a promising natural alternative to synthetic colorants, with applications in the food and beverage industry. The antioxidant properties of beetroot pigments are attributed to their ability to scavenge free radicals, making them suitable for incorporation into functional foods and dietary supplements.

● **Benefits of Beetroot Juice**

1. Rich in Nutrients and Bioactive Compounds

- Beetroot juice is packed with vitamins (like vitamin C), minerals (like potassium and magnesium), and antioxidants such as betalains. These nutrients create a favorable environment for the growth of beneficial gut bacteria.

2. Prebiotic Potential

- Beetroot contains dietary nitrates and fiber that can act as prebiotics, feeding and supporting the growth of probiotics (beneficial bacteria) in the gut.

3. Enhanced Gut Microbiota Diversity

- Regular consumption of beetroot juice may encourage the proliferation of beneficial bacteria, contributing to a balanced and diverse gut microbiota.

4. Anti-inflammatory Properties

- The betalains in beetroot juice have anti-inflammatory effects, which may help maintain gut lining integrity and reduce inflammation-related gut issues.

5. Improved Digestion

- By supporting beneficial bacteria and gut health, beetroot juice can improve digestion and nutrient absorption.

6. Support for Probiotic Supplements

- When consumed alongside probiotic-rich foods or supplements, beetroot juice can enhance the survival and activity of probiotics by providing them with nutrients.



7. Boosts Nitric Oxide Production

- The nitrates in beetroot juice are converted into nitric oxide by gut bacteria. Nitric oxide improves blood flow, potentially benefiting intestinal health.

8. Antimicrobial Effects Against Harmful Bacteria

- Beetroot juice may inhibit harmful bacteria due to its antimicrobial properties, creating a healthier environment in the gut for probiotics to thrive.

9. Detoxification Support

- Beetroot juice enhances liver detoxification processes, indirectly supporting gut health and probiotics by reducing the toxin load in the digestive system.

Potential Health Benefits Gut Health: Probiotics can help balance gut microbiota, improving digestion and nutrient absorption. **Blood Pressure Regulation :**The nitrates in beetroot may help lower blood pressure, especially when combined with probiotics .**Overall Wellness :**Regular consumption may enhance immune function and reduce inflammation.

Beetroot juice as a prebiotic offers several advantages:

1. **Nutrient Density Vitamins and Minerals :** Rich in vitamins A, C, and B6, along with minerals like iron and potassium, which support overall health.
2. **Antioxidant Properties High in Antioxidants:** Contains betalains, which help combat oxidative stress and inflammation.
3. **Enhanced Digestive Health Gut Microbiota Balance:** Prebiotics in beetroot juice can improve gut health by promoting beneficial bacteria.
4. **Blood Pressure Regulation Nitrate Content:** Beetroot juice is high in dietary nitrates, which can help lower blood pressure and improve circulation.
5. **Improved Nutrient Absorption Digestive Support:** Prebiotics can enhance the absorption of nutrients from food.

The stability of beetroot juice as a probiotic involves several factors that influence the viability of probiotics and the overall quality of the juice over time. Here are key aspects to consider:

1. **Microbial Viability Storage Conditions:** Probiotic stability is significantly affected by temperature. Refrigeration can help maintain viable cell counts, while higher temperatures can lead to a rapid decline in probiotics .**pH Levels:** The acidity of the juice can influence probiotic survival. A lower pH generally favors the growth of beneficial bacteria but may affect the taste.
2. **Nutritional Composition Sugar Content:** High sugar levels can serve as a food source for probiotics, enhancing their growth. However, excessive sugar can lead to fermentation that alters flavors and quality. **Antioxidant Presence:** The natural antioxidants in beetroot can help protect probiotics from oxidative stress, potentially extending their viability.
3. **Packaging Oxygen Exposure:** Use airtight, opaque containers to minimize exposure to oxygen and light, which can degrade both probiotics and juice quality. **Materials:** Choosing suitable packaging materials can prevent contamination and maintain stability

Fermentation Process Duration and Conditions: The fermentation time and conditions (temperature, inoculation rate) can affect both the flavor and probiotic content of the juice.

□ **What is beet juice good for?**

Beet juice is an excellent source of potassium and essential minerals like iron and manganese. Drinking beet juice consistently may help: lower blood pressure reduce LDL or “bad” cholesterol improve exercise stamina improve muscle strength in people with heart failure you maintain a healthy weight prevent or reduce fatty deposits from forming in your liver support overall health during chemotherapy.



❖ **Effect of Beetroot on the Microbiota:**

Red beetroot, which is rich in betanin, has the potential to function as a prebiotic and influence gut microbiota. Since each individual's gut microbiota is unique, the effectiveness of prebiotics can vary depending on their enterotype. This study proposes that the impact of red beetroot may differ accordingly. The human intestine is home to trillions of microorganisms, often referred to as the "second genome." These gut microbes play a crucial role in overall health by aiding digestion and nutrient absorption. They are also linked to various metabolic conditions, including obesity, hypertension, diabetes, allergies, and depression. The gut microbiota contributes to immune function, breaks down food to release nutrients, and produces essential metabolites such as amino acids, short-chain fatty acids (SCFAs), and vitamins. SCFAs, generated through the fermentation of dietary fibers and non-digestible carbohydrates, help strengthen the immune system, increase resistance to infections and inflammation, and act as signaling molecules for the nervous and endocrine systems. The composition of gut microbiota is closely related to SCFA production, with specific bacterial strains such as *Faecalibacterium*, *Bifidobacterium*, and *Bacteroides* being particularly efficient at fermenting dietary fibers and producing SCFAs.

Several factors influence gut microbiota composition, including age, diet, and geographical location. The concept of enterotypes categorizes gut microbiota into distinct groups based on core bacterial genera such as *Bacteroides*, *Prevotella*, *Ruminococcus*, *Bifidobacterium*, and *Faecalibacterium*. Red beetroot (*Beta vulgaris* L.) is widely consumed for its health benefits due to its high content of phytochemicals, including dietary fiber, polyphenols, and betalains. Betanin, the primary betalain in red beetroot, is classified under the betacyanin subgroup and is the only one approved as a food additive by the European Union and the U.S. Food and Drug Administration (FDA). Betanin is known for its antioxidant, anti-inflammatory, and immune-boosting properties, which may be influenced by gut microbiota. Gut bacteria produce enzymes such as β -glucosidase and glycoside hydrolases, which help metabolize these phytochemicals, including dietary fibers, further enhancing their health benefits.

❖ **How Does beet juice detox your body :**

Your body naturally detoxifies without a specific diet, such as beetroot juice. Your kidney, liver, digestive system, and other organs typically work together to remove toxins.

However, beet juice contains beneficial nutrients that may support your overall health along with a balanced diet.



Fig.No 2

Beetroot :

Beetroot is a nutrient-rich vegetable containing carbohydrates, fibers, proteins, and essential minerals like sodium, potassium, calcium, and iron . It also produces geosmin, a bicyclic alcohol responsible for its distinctive "earthy" taste. To eliminate this undesirable flavor, a distillation process is utilized during juice concentration.

Raw beetroot is composed of 88% water, 10% carbohydrates, 2% protein, and less than 1% fat . A 100-gram (3.5-ounce) serving delivers 180 kilojoules (43 kilocalories) of energy and is an excellent source of folate, providing 27% of the Daily Value (DV). It is also a moderate source of manganese (16% DV), while other nutrients are present in negligible amounts.



Fig no:3

❖ Drug Profile:

Biological Source of Beetroot

Botanical Name: Beta vulgaris L.

Family: Amaranthaceae (formerly classified under Chenopodiaceae).

Part Used: Root (commonly), leaves (occasionally used).

Habitat: Native to the Mediterranean region but widely cultivated globally in temperate climates.

Description: Beetroot is a biennial herbaceous plant with a swollen, fleshy taproot and leafy greens.

Varieties:

1. Table Beet: Commonly used for food and juice.
2. Sugar Beet: Cultivated for sugar extraction.
3. Fodder Beet: Used as animal feed.
4. Leaf Beet: Cultivated for its leaves (e.g., Swiss chard).

Chemical Constituents

Betalains: Betacyanins (red pigments) and betaxanthins (yellow pigments).

Nitrates: High concentration, converted into nitric oxide in the body.

Carbohydrates: Sucrose as the primary sugar.

Vitamins: Folate, Vitamin C.

Minerals: Potassium, iron, magnesium, and manganese. This biological source makes beetroot a versatile crop with applications in food, medicine, and industry.

MATERIALS AND METHODS:

A. Sample Procurement and Preparation

Fresh beetroot was procured from the local market, thoroughly cleaned, and stored at 4°C for subsequent use. Juice was extracted using a food processor equipped with a juicer. The extracted juice was then filtered and pasteurized at 80°C for 10 minutes. After pasteurization, the juice was cooled to room temperature to prepare it for microbial inoculation.

B. Inoculum Preparation

Probiotic strains of *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus delbrueckii* subsp. were procured from IMTECH, Chandigarh. To prepare the inoculum, glycerol stock cultures of these microorganisms were transferred into 250 mL Erlenmeyer flasks containing 100 mL of MRS broth. The cultures were incubated at 37°C to promote bacterial growth. The growth was monitored using a spectrophotometer at 590 nm, and incubation was continued until the optical density (OD) reached 0.600, equivalent to a cell density of 9.00 Log CFU/mL as per the McFarland standard

C. Inoculation and Fermentation:

The optimal fermentation conditions were determined using a Central Composite Rotated Design (CCRD), with a pH range of 4–7 and a temperature of 37°C. These parameters were chosen because they support the growth of *Lactobacillus* species. The pH of clarified beetroot juice was adjusted to the desired level using 0.1 N HCl. A pre-calculated concentration of inoculum was then introduced to the beetroot juice, ensuring a final concentration of 7.00 Log CFU/mL, as recommended for probiotic foods. This was achieved by adding 1 mL of MRS broth containing 9.00 Log CFU/mL of *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus delbrueckii* subsp. Fermentation was carried out under static conditions in an incubator at 37°C for 24 hours.

D. Sample Analysis

Fresh and probiotic beetroot juice were evaluated for protein content, acidity, pH, total soluble solids (TSS), and betalain content using various analytical methods. Additionally, bile and acid tolerance were assessed prior to the introduction of lactic acid bacteria.

E. Growth of Probiotic Strains

Using the optimized conditions (temperature of 37°C and pH 6.5), cultures were grown in

pasteurized beetroot juice (700 mL in a 1 L plastic flask) with the addition of lactic acid bacteria inoculum at concentrations of 2.5%, 5%, and 7% (v/v). The cultures were incubated at 37°C for 24 hours. Growth and viable cell counts were monitored every two hours. Microbial growth was evaluated by observing changes in pH, acidity, and sugar utilization. pH was measured using a pH meter, acidity was determined by titration, and sugar content was assessed using the DNS (dinitrosalicylic acid) method.

□ Evaluation Parameters

■ Sensory Evaluation

Sensory evaluation was performed using semi-trained panelists, employing a nine-point hedonic scale ranging from "liked extremely" to "disliked extremely", as outlined by Larmond. The samples were assessed based on various attributes including appearance, aroma, consistency, taste, mouthfeel, and overall acceptability.

■ Statistical Analysis

The physico-chemical, microbiological, and sensory properties of papaya-based whey RTS were assessed using GraphPad Prism software (version 5.01, La Jolla, CA, USA). A two-way ANOVA was performed to determine the statistical significance of mean differences, with a significance level set at 5% ($P < 0.05$) for all analyses.

■ Total Phenolic Content (TPC)

The total phenolic content was measured using the Folin-Ciocalteu reagent, following the method described by Lanzerstorfer et al. (2014). In brief, the samples were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was used for quantifying the total phenolic content. A mixture was prepared by combining 1.05 mL of distilled deionized water, 12.5 μ L of the sample supernatant, and 62.5 μ L of Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 3–6 minutes, after which 125 μ L of sodium bicarbonate solution (200 g/L) was



added. The samples were incubated for 60 minutes at room temperature in the dark, and absorbance was measured at 720 nm. The total phenolic content was expressed as (+)-catechin equivalents in mg/L of the sample. Each juice sample was measured in triplicate.

■ Quantitation of Betalain Content

Betalain content was quantified for each sample using the method described by Stintzing et al. (2003). The total concentration of betalains was determined by adding the concentrations of betacyanins and betaxanthins. In brief, the absorption of betacyanins was measured at 536 nm, and the absorption of betaxanthins was measured at 485 nm. The concentrations were calculated using the following formula Betacyanins (betaxanthins) content (in mg/L) = $\frac{A_{DF} \times MW}{\epsilon \times I} \times 100$ where A = $A_{536nm} - A_{650nm}$ (for betacyanins) or $A_{485nm} - A_{650nm}$ (for betaxanthins); DF = dilution factor; MW (molecular weight) = 550 g/mol (for betacyanins) or 339 g/mol (for betaxanthins); $\epsilon = 60,000$ (molar extinction coefficient in $L \times mol^{-1} \times cm^{-1}$ for betacyanins) or 48,000 (for betaxanthins); i = path length (cm). The samples were pre-diluted with distilled deionized water and measured in triplicate

CONCLUSION:

Beetroot juice demonstrates significant potential as a functional food for supporting gut health through its prebiotic properties and synergistic effects with probiotics. Rich in bioactive compounds, it promotes the growth of beneficial gut microbiota, enhances microbial diversity, and contributes to improved gut barrier integrity and overall metabolic health. Emerging evidence suggests that beetroot juice could amplify the efficacy of probiotic strains, such as *Lactobacillus* and *Bifidobacterium*, further highlighting its role in modulating gut microbiota composition and function.

RESULTS:

The review highlights a growing body of evidence supporting the potential of beetroot juice to influence gut microbiota and enhance probiotic function. While the results are promising, significant variability exists in the methodologies used across studies, including differences in beetroot juice preparation, dosing and study populations.

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