Characterization of Langra Mango Peel Powder and Assessment of Its Prebiotic and Antioxidant Potential

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Received 7 December 2023; Revised 2 March 2024; Accepted 7 March 2024; Published 21 March 2024

The possibility of developing waste by-products of food processing into functional food additives along with probiotics is an interesting avenue to research. This study investigated the nutritional and functional attributes of dried mango peel powder (MPP) of Langra cultivar and its putative potential to act as a prebiotic in the presence of two probiotic strains Lactobacillus rhamnosus NCDC347 and Limosilactobacillus fermentum NCDC143@2.5 & 5% after 24 to 48 h fermentation. Proximate analysis revealed that the MPP contains 6.45 % moisture, 6.34 % protein, 3.88 % fat, 2.50 % ash, 32.86 % crude dietary fiber, and 47.97 % of total carbohydrate content. MP displayed substantial antioxidant potential with 54.6 % DPPH inhibitory activity, 15.67 mg GAE/g TPC, 8.88 mg QuE/g TFC, OHC of 1.47 g oil/g, and a WHC of 4.7 g water/g. MPP could selectively stimulate the growth of two probiotic strains over enteric bacteria. It was revealed that a combination of MPP @5% with L. fermentum NCDC143 after 24 h fermentation had the best in vitro prebiotic activity score of 3.35 and 3.53 against Escherichia coli ATCC 25922 and Enterococcus faecalis NCDC114, respectively. The prebiotic activity score of MPP was better than commercial prebiotic malto-dextrin for all combinations of probiotic and enteric strains tested. The percentage DPPH inhibition activity of MPP increased during fermentation with L. fermentum NCDC143, highlighting its role as a source of antioxidants. These findings contribute to the formulation of synbiotic products that are able to maintain selected healthy microbiota in the human gut.

1. Introduction

Mango (Mangifera indica L.), a class of drupe fruits, belongs to the Anacardiaceae family. Mango fruit from different varieties varies in shape, color, taste, and flesh texture and is often termed the king of fruits due to its relishing taste, aroma, and nutritional value [1, 2]. The genus Mangifera originated in tropical Asia and is a commercially important crop in India. India led the globe in mango production, as it produced 26.3 MT of mangoes, followed by Indonesia (4.1 MT), China and China mainland (4 MT and 3.8 MT), and Pakistan (2.8 MT) in the year 2022 [3]. India is renowned for its wide variety of mangoes, including well-known kinds like Alphonso, Kesar, Totapuri, Langra, Fazli, Dasheri, etc. The Langra mango is a popular cultivar known for its unique taste, juicy, sweet flesh, intense aroma, and thin sheath famous in northern India and Pakistan [4–6]. Langra variety of mango is one of India’s most sought-after
varieties of mangoes, and it was recently tagged with the geographical indication (GI) for the Varanasi district of Northern India.

Mango is a seasonal fruit; hence, 20% of the total harvest is processed to make products widely used worldwide, including puree, juice, squash, jam, dried powder, leather, pickles, and canned slices [7, 8]. Pulp, which is approximately 33–85% of the whole mango fruit, is generally consumed, while the kernel and peel, representing almost 9–40% and 7–24% of mango, respectively, are discarded directly [9]. These mango by-products constitute a severe disposal problem owing to their high biological oxygen demand (BOD). Utilization of by-products from food industries is one of the most challenging global issues [10]. However, on the brighter side, these by-products are being utilized to produce valuable biomolecules via microbial fermentation or enzymatic treatment for biofuels, bioplastics, biotherapeutics, biopreservatives, and nutraceuticals [10–12]. The by-products from the fruit and vegetable industry are an excellent source of dietary fibers (DF) and polyphenols (PP), flavoring compounds, and vitamins [13]. Hence, it is of immense interest to explore how these active ingredients can be extracted and used as additives in nutraceuticals and functional foods [14]. From 2022 to 2029, the demand for bioactive compounds is anticipated to increase at a faster pace because of the widespread awareness of nutrition and health and the consumption of functional foods due to the COVID-19 pandemic and other diseases [15, 16].

Mango peels are also a rich source of dietary fiber, an essential functional ingredient, containing 29–50% insoluble and 16–28% soluble dietary fibers, depending on the variety [17]. DF is defined as a nonstarch polysaccharide obtained from edible plant components, not completely digested by gastric enzymes, and thus reaching the colon [18]. After reaching the colon, some DF may be fermented by gut microbiota and form short-chain fatty acids (SCFA) like butyrate, acetate, and propionate [19, 20]. Fermentation of DF and production of SCFAs and other metabolites may lead to certain alterations in gut microbiota composition and their overall activities in the gut, conferring health benefits to the host. In such cases, the specific DF is considered to be a prebiotic [21, 22]. The consumption of prebiotics is associated with a reduced risk of colon cancer and heart disease [23].

The peel of mango fruit possesses up to 100 mg/g of polyphenolic compounds, including flavonoids, alkylresorcinols, hydrolyzable tannins, and proanthocyanidins [24]. Polyphenols exhibit various beneficial health effects, including antioxidant, antidiabetic, neuroprotective, and antitumor properties [25]. Another important class of bioactive compounds found in mango peel is the carotenoids [26]. Certain minerals such as Na, Fe, Ca, Zn, Cu, Mg, Mn, and K are in higher amounts in the peel of mango rather than in its pulp [14]. Thus, this overlooked part of the fruit holds the potential to offer numerous health benefits and culinary applications besides having a sustainable environment. Unveiling the hidden potential of mango peel can lead to a greater appreciation of this tropical gem and contribute to sustainable practices in fruit consumption and processing.

Recently, the extraction and value-addition of these carotenoids from agroindustrial by-products have become immensely popular. The recovery of DF and bioactive polyphenols could be an economically feasible option for the development of potential prebiotics and antioxidants for their use in the food and pharmaceutical industries. A few investigators have studied the potential of mango by-products as a novel source of prebiotics [27, 28]. Mango peels have been used as functional food ingredients in novel ready-made formulations such as bakery and confectionery food items [29, 30]. Researchers have studied the effect of the phenolic chemicals from mango by-products and their ability to alter the gut flora [31–33] positively.

Recently, huge interest has been felt in research studies on exploring local natural resources for macromolecules with putative functional potential to be developed into commercially valuable products to benefit local stakeholders of one area. Bioprospecting natural resources or waste by-products of the food industry helps maintain a sustainable environment and indicates better entrepreneurial opportunities for the increasing population. Scanty information is available on the prebiotic potential of Langra mango peel. It is not utilized to its full potential and is generally discarded as agrowaste. Hence, the present study was designed to determine the nutritional content, phytochemical composition, and functional characteristics of Langra mango peel powder and to investigate its suitability as a potential prebiotic candidate for specific probiotics Lactobacillus rhamnosus NCDC347 and Limosilactobacillus fermentum NCDC143.

2. Materials and Methods

2.1. Langra Mango and Microbial Cultures. Optimally ripened mangoes (cultivar Langra) were purchased from a local fruit market located in Sunderpur, Varanasi, Uttar Pradesh, India. Freeze-dried vials of probiotic strains Lactobacillus rhamnosus NCDC347 (LGG) and Limosilactobacillus fermentum NCDC143 (LF) were procured from the National Collection of Dairy Cultures, National Dairy Research Institute, Karnal, Haryana, India. Enteric Gram-negative bacterial cultures of Escherichia coli ATCC 25922 and Enterococcus faecalis NCDC114 were kindly donated by the Dairy Microbiology Division, NDRI, Karnal, Haryana.

2.2. Chemicals and Reagents. All chemicals were analytical-grade chemicals from Himedia Pvt. Ltd., Mumbai. The glassware and plastic ware used in the study were purchased from Borosil, India, and Tarsons Pvt. Ltd., respectively.

2.3. Preparation of Mango Peel Powder (MPP). The mangoes were thoroughly washed in running water and were left to surface dry at ambient temperature. Subsequently, the mango peel was removed using a peeler. These peels were then weighed on a scale and evenly spread in drying trays before being placed in a tray dryer set at 50°C for 18 hours. Afterward, the peels were ground into a powder using a grinder and then sieved through a 250 μm mesh to ensure...
a homogeneous particle size. The resulting powder was sealed in airtight bags and stored in a desiccator for further analysis.

2.4. Proximate Composition Analysis of MPP. Moisture, crude fat, crude protein, total dietary fiber (TDF), and total ash were analyzed following the AOAC methods (AOAC, 2000). Briefly, 5 g of MPP was taken in triplicates and placed in clean, dry, and preweighed silica dishes and kept in a hot air oven (PS oven, Perfect Solutions Limited, India) at 105°C for approx. 24 h (until the weight was stable). The moisture content was determined as a percentage. The silica dishes containing the oven-dried sample were ignited over a flame until they ceased smoking to burn off the organic matter. After charring, the silica crucibles were placed in a muffle furnace (SNOL 8, 2/1100-1LZ Pagaminta Lietuvoje, Lithuania) and heated to 550°C for 5–6 hours or longer until the content was determined as a percentage. The silica dishes containing the oven-dried sample were ignited over a flame until they ceased smoking to burn off the organic matter. After charring, the silica crucibles were placed in a muffle furnace (SNOL 8, 2/1100-1LZ Pagaminta Lietuvoje, Lithuania) and heated to 550°C for 5–6 hours or longer until the sample was reduced to grayish or off-white ash. The crucibles were weighed immediately after cooling in a desiccator, and the percentage of ash was calculated.

The Soxhlet technique estimated total fat content. Briefly, two grams of sample were taken in a thimble and placed in a clean, dried, and previously weighed Soxhlet beaker. The beakers were carefully placed in the extractor. The extractor was filled with petroleum ether, and the top inlet was plugged with cotton to prevent the solvent from escaping. The Soxhlet apparatus (SOCS PLUS, SCS-4 Chennai, India) was switched on at a temperature of 70°C inlet was plugged with cotton to prevent the solvent from escaping. The Soxhlet apparatus (SOCS PLUS, SCS-4 Chennai, India) was switched on at a temperature of 70°C for 2 hours for extraction. Following the extraction process, the temperature was raised to 140°C for 10 minutes for the complete removal of moisture and solvent. The beakers were carefully removed and placed in a desiccator for cooling, after which they were weighed, and the percentage of fat was calculated.

The crude protein in MPP was determined using the Kjeldahl method along with control. The sample (0.1–0.2 g) was weighed and placed in a digestion tube with a 5 g digestion mixture (made by mixing CuSO₄ and K₂SO₄ in a 1:8 ratio) and 10 ml of conc. sulfuric acid. This mixture was digested at 420°C until it became colorless. The sample was cooled and dissolved in deionized water in a volumetric flask. During distillation, a flask containing 10 ml of 4% aqueous solution of boric acid with a few drops of Tashiro’s indicator (pink in color) was positioned at the receiving end of the distillation setup, with the condenser’s tip slightly immersed in boric acid. An aliquot of the digested sample was pipetted from the flask into the distillation unit. Distilled water (10–20 ml) was added, followed by 40–60 ml of 40% NaOH into the tube. The contents were steam distilled for about 4-5 minutes until a faint green color was obtained.

Finally, the solution in the receiving flask was collected and titrated with N/100 sulfuric acid until the original pink color reappeared. The volume of acid used for titration was recorded. This distillation and titration process was repeated three times to obtain a consistent value. Nitrogen content was quantitated, and total protein was expressed using 6.25 as the conversion factor.

Total dietary fiber (TDF) was estimated using the gravimetric enzymatic method. One-gram sample was digested with amyloglucosidase α-amyrase and protease for the removal of starch and protein. Ethanol was employed to precipitate soluble fiber content, and the resultant solid was filtered and rinsed. After drying, the samples were weighed and examined for their ash and protein content. The total dietary fiber (TDF) was determined by subtracting the combined weight of protein and ash from the weight of the residue and expressing it as a percentage of the original sample weight. The total carbohydrate content was estimated as 100 minus the sum of the percentages of crude protein, crude fat, total ash, total dietary fiber, and moisture in the sample.

2.5. Determination of Antioxidant Properties of MPP

2.5.1. Preparation of Extract. Two grams of the sample were added to 20 ml of 80% methanol solution, followed by proper extraction in a shaking incubator (Infors HT Ecolab, UK) at ambient temperature for 2 h at 100 rpm. A clear supernatant was obtained by centrifuging the extract at 6000 rpm for 20 min at 4°C. The supernatant was then filtered from Whatman® paper 1, followed by filtration through a 0.22 μm syringe filter. The filtrate was stored at 4°C until used for further analysis.

2.5.2. Diphenyl-2-Picrylhydrazyl (DPPH) Inhibitory Activity. The antioxidant activity of the MPP was determined using the DPPH radical scavenging assay method according to [34, 35] with slight modifications. DPPH stock solution of 0.1 mM in methanol was prepared. 700 μl of the prepared extract was added to 700 μl of DPPH solution and mixed properly by shaking in a vortex machine (MSW-308, Deluxe Model, Macro Scientific Works Pvt. Ltd., Delhi, India.). Absorbance was measured using a spectrophotometer (UV-1800 Shimadzu Corporation, Japan) at a wavelength of 517 nm after leaving the mixture at room temperature in the dark for 30 min, along with a control sample (methanol). The antioxidant activity of the MPP was determined as DPPH inhibitory activity in percentage by the following expression:

\[
\text{DPPH Inhibitory Activity (\%)} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100. \tag{1}
\]
2.8. Termo-Gravimetric Analysis (TGA) of MPP. An aluminum pan on the platinum basket in the TGA chamber, (PerkinElmer, Singapore) was used. The specimen was first put in a simultaneous Thermal Analyzer (STA) 6000 (PerkinElmer, Germany) at 4°C. The supernatant was decanted, and 3000g for 20min using a refrigerated centrifuge (3–30K rpm). The resultant solution was kept for incubation at room temperature after which the absorbance was taken at 750 nm wavelength. Results were compared with the absorbance of a standard gallic acid curve (0–500 μg/mL). Results were expressed as mg gallic acid equivalent (GAE)/g of MPP.

2.10. Assessment of the Prebiotic Effect of MPP. Freeze-dried vials of Lacticaseibacillus rhamnosus NCDC347 and Limosilactobacillus fermentum NCDC143 were opened aseptically, and their contents were transferred to sterilized deMan Ragosa Sharpe (MRS) broth. The MRS tubes were then incubated at 35 ± 2°C for 24 hours. Both strains were propagated at a 1% rate, twice from the initial MRS tubes, at 35 ± 2°C for 18 hours. Gram-negative strains Escherichia coli ATCC 25922 and Enterococcus faecalis NCDC114 were inoculated at a 1% rate in sterile BHI broth tubes and incubated at 37°C for 24 hours. The purity of cultures was checked by Gram staining and catalase test. Young cultures with 8–9 hours of growth were used to prepare 25% glycerol stocks for maintaining the cultures at −20°C. These microbial strains were freshly propagated twice before any microbial analysis.

MRS and BHI broth tubes were autoclaved after supplementation with mango peel powder (MPP) and malto-dextrin (MD) separately at 2.5% and 5% concentrations and 1% glucose as controls. In MRS tubes, Lacticaseibacillus rhamnosus NCDC347 and Limosilactobacillus fermentum NCDC143 were inoculated at 1%, incubated at 35 ± 2°C, and samples were taken at 0, 24, and 48 hours. Serial dilutions were plated on MRS agar for viable count determination. In BHI tubes, Escherichia coli ATCC 25922 and Enterococcus faecalis NCDC114 strains were inoculated at 1%, incubated at 37°C, and sampled at 0, 24, and 48 hours. Serial dilutions were plated on BHI agar for viable count determination. The same procedure was repeated with controls (only broth tubes with 1% glucose) for both MPP and MD supplementation, providing insights into the growth dynamics of probiotics and pathogenic strains with these substrates [40].

2.11. Determination of Prebiotic Activity Score of MPP. The in vitro prebiotic activity score was calculated to evaluate the ability of MPP and MD as substrates to stimulate the growth of selected probiotic strains relative to that of a nonprebiotic substrate with respect to the growth of the selected enteric bacteria [40]. The prebiotic activity score was determined for both MPP and MD by using the following equation:
Prebiotic activity score = \frac{(\text{Probiotic (LogCFU/mL) on the prebiotic at 24 h}) - (\text{Probiotic (LogCFU/mL) on the prebiotic at 0 h})}{(\text{Probiotic (LogCFU/mL) on the control at 24 h}) - (\text{Probiotic (LogCFU/mL) on the control at 0 h})}
- \frac{(\text{Enteric (logCFU/mL) on the prebiotic at 24 h}) - (\text{Enteric (logCFU/mL) on the prebiotic at 0 h})}{(\text{Enteric (LogCFU/mL) on the control at 24 h}) - (\text{Enteric (LogCFU/mL) on the control at 0 h})}

2.12. Antioxidant Activity (% DPPH Scavenging Assay) of the Probiotic Cultures in Combination with MPP. The antioxidant potential of the MPP as a substrate for probiotic strains during 48 hours of incubation at 35 ± 2°C was evaluated. % DPPH scavenging assay of various concentrations of MPP (0%, 2.5%, and 5%) inoculated with probiotic cultures was determined at different points of time (0, 24, and 48 h), following the method given in Section 2.5.2.

2.13. Statistical Analysis. A general linear model analysis of variance (ANOVA) was performed to assess the impact of various groups formed by MPP and its concentrations on the bacterial counts. The compositional and antioxidant assessments of MPP were analyzed by one-way ANOVA. Tukey’s test was employed to distinguish means among different treatments with a 95% confidence level. All statistical analyses were performed using the IBM® SPSS® Statistics version 29 and Graph Pad Prism version 5.

3. Results and Discussion

3.1. Proximate Analysis of Mango Peel Powder (Langra Variety). The colour of the dried mango peels was a mixture of brown, green, and yellow. Determining the proximate composition is crucial in evaluating the quality and potential functional attributes of the sample. The proximate composition of mango peel powder (MPP) is presented in Table 1. The mean moisture content of oven-dried MPP was 6.45 ± 0.20%, which was slightly lower than the value (8.26 ± 0.24%) reported by [41] for an unnamed variety of MPP. Dried powders and similar products, given their minimal moisture content, tend to possess a long shelf life, thereby reducing the risk of microbial decay or chemical alterations due to the low \(a_w\) associated with such low levels of moisture.

The crude protein content (6.34 ± 0.16%) and the crude fat content (3.88 ± 0.06%) in MPP were comparable with the amounts (6.55 ± 0.32% and 3.66 ± 0.12%, respectively) obtained by [41]. The ash content of a sample refers to the residual inorganic material left behind after the complete combustion or incineration of the organic components. The ash content of the MPP (2.50 ± 0.10%) was seemingly lower than the value (3.43 ± 0.22%) obtained by [41]. However, the ash content was comparable to the value (2.21 ± 0.19%) reported by [42] for Langra mango. The variations in the ash content could be due to the differences among cultivars, ripening stages, and climatic conditions.

The crude dietary fiber in MPP (32.86 ± 1.11%) was comparable to the amount (29.83 ± 0.12%) obtained by [41]. Plant-derived fibers constitute the structural component of cell walls and predominantly consist of polysaccharides and oligosaccharides such as cellulose and hemicellulose. These fibers are renowned for their positive impact on health [43]. Furthermore, the substantial fiber content, as identified in the tested MPP, holds significant potential in the food industry. For example, incorporating fiber may enhance the yield, water retention, and viscosity in foods like minced meat blends and soups. Additionally, it can improve the texture of baked goods such as bread and cookies [44].

The total carbohydrate content found in MPP (47.97 ± 2.52%) closely resembled the carbohydrate percentage (52.3 ± 0.6%) observed in the mango peel of cultivar “sugar” as reported by [45]. It is crucial to highlight that the carbohydrate levels were determined using the subtraction method as outlined in Section 2.4, and the fiber analysis was conducted and expressed as total dietary fiber. Consequently, the calculated carbohydrate values represent only soluble sugars like glucose, fructose, etc.

Proximate analysis indicated that MPP is an excellent source of dietary fibers and carbohydrates while being a limited source of proteins and fats. Both the dietary fibers and carbohydrate components within the MPP might act as substrates for microorganisms, potentially producing short-chain fatty acids and contributing to the development of a healthier gut microbiota.

3.2. Characterization of Mango Peel Powder (Langra Variety). The functional groups in the Langra mango peel powder were determined by FTIR spectral in the midinfrared region (4000–600 cm\(^{-1}\)) (Figure 1). The absorption band at 3300 cm\(^{-1}\) was a typical representation of the O–H of carboxylic acid suggesting strong hydrogen bonding in molecules of phenolic compounds [46]. The absorption bands around 2917 cm\(^{-1}\) indicate the C–H stretching of the CH\(_2\) groups of aliphatic compounds [47]. In between the 1719–1600 cm\(^{-1}\) region, the sharp absorption peaks indicate the carbonyl (C=O) stretching of carbonyl compounds. The presence of carbonyl functional groups indicates high concentration of flavonoid compounds. The absorption bands at 1439 cm\(^{-1}\) indicate that the C–C–O stretching vibration and O–H bending vibration were reflected by absorption bands between 1315 and 1142 cm\(^{-1}\). These typical absorption bands are indicative of the presence of phenolic compounds such as quercetin, rutin, and tannic acid [46]. An absorption band at 1020 cm\(^{-1}\) corresponds to the C–O functional group of compounds. The absorption bands from 874 to 762 cm\(^{-1}\) are typical of phenolic compounds [48].
3.3. Thermogravimetric Analysis of Mango Peel Powder (Langra Variety). The mango peel powder was thermalized at various heating estimates (Figure 2). Thermal stability of the peel and its powers are crucial aspects when the peel powders are used in high heat-treated food products such as baked items [29] or UHT treated dairy or nondairy beverages or in cases where peel powders are used to manufacture biocomposite films [49] or other semi-synthetic packaging materials [50]. Initial decomposition (~100°C) occurred due to water evaporation, followed by significant weight loss (~200°C) due to pyrolytic degradation of polysaccharides in mango peel powder. In the final stage, there is a weight reduction attributed to the thermal degradation and depolymerization of lignin and hemicellulose. No weight loss was observed at around 700°C. Comparable thermal degradation pattern was observed by [51].

3.4. Scanning Electron Microscopy Analysis (SEM). SEM analysis of mango peel powder was done to understand the morphological features (Figure 3). It is visible that the granules possess solid structures of various sizes and irregular amorphous forms. The amorphous structures have an uneven appearance, rough texture, and fibrous surface that could be caused by microstructural damage to the cell walls from water loss and component segregation during drying [52]. Drying procedures frequently alter the microstructure of peels, making them stiff and damaging the cellular tissue [53].

3.5. Antioxidant Potential of Langra Mango Peel Powder. The polyphenolic contents and antioxidant potential of the MPP (Langra variety) have been summarized in Table 2. Mango peel powder (Langra variety) in this study possessed...
Previous studies have reported that mango peel powder from different cultivars had a varying range of phenolic compound content, i.e., from 14.85 to 127.6 mg/g of MPP dry weight basis [14, 54, 55]. The results obtained in this study are significantly different from the previous studies on the Langra cultivar, where it was 15.67 ± 0.15 mg/g TPC content. The figures show thermal decomposition and SEM images of mango peel powder.
Table 2: Antioxidant potential and water and oil holding capacity of Langra mango peel powder.

<table>
<thead>
<tr>
<th>Functional properties</th>
<th>Average values*</th>
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<tbody>
<tr>
<td>Total polyphenol content</td>
<td>15.67 ± 0.15 mg GAE/g</td>
</tr>
<tr>
<td>Total flavonoid content</td>
<td>8.33 ± 0.13 mg QuE/g</td>
</tr>
<tr>
<td>% DPPH inhibitory activity</td>
<td>54.6 ± 2.86%</td>
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<tr>
<td>Water holding capacity</td>
<td>4.7 ± 0.02 g water/g</td>
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<tr>
<td>Oil holding capacity</td>
<td>1.47 ± 0.02 g oil/g</td>
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*Values are demonstrated as the mean ± standard error for triplicate samples.

3.6. Oil and Water Holding Capacity of MPP. Oil and water holding capacities are investigated to determine the technofunctional aspects of food ingredients that play a vital role in new product development. The water holding capacity was found to be 4.7 ± 0.02 g water/g sample, and the oil holding capacity was found to be 1.47 ± 0.02 g oil/g for the MPP sample, and the difference between WHC and OHC of the MPP in this study is significant (p < 0.05). The WHC represents the weight of the water held per unit mass of the sample [60]. WHC of any substance indicates the amount of fiber content present in it that can hold water molecules [28, 41, 60]. The result of the WHC of MPP was similar to the results of the study conducted by [60] and was comparatively higher, as reported by [28, 41].

The value of OHC was in close proximity to the results of [28]. Since MPP exhibited optimum WHC and OHC, they can serve a dual role as both hydrophilic and hydrophobic agents. This versatility may allow the Langra MPP to be used as a low-cost emulsifier in food products.

3.7. Determination of Selective Growth Stimulation of Probiotic Bacteria on Mango Peel Powder. The efficacy of MPP as a potential prebiotic in stimulating the growth of two different probiotic strains was determined. Figure 4 represents the effect of different concentrations of MPP (0%, 2.5%, and 5%) supplemented in MRS broth as culture media on the growth of (A) Lactocaseibacillus rhamnosus NCDC347 (LGG) and (B) Limosilactobacillus fermentum NCDC143 (LF) during 48 hours’ incubation at 35 ± 2°C. The results show a significant (p < 0.05) increase in the number of both probiotic strains used. The LGG log counts increased by 3.311, 4.965, and 5.521 log CFU/ml for concentrations 0% (control), 2.5%, and 5%, respectively, after 24 h incubation time. The percentage increase in log CFU/ml for control, 2.5%, and 5% MPP concentrations were 52.48%, 81.60%, and 78.40%, respectively, from 0 h to 24 h incubation period. This indicates the efficacy of MPP to stimulate the growth of LGG significantly. Similarly, LF log counts also increased by 1.668, 3.507, and 5.521 log CFU/ml with the percentage increase of 26.19%, 52.98%, and 93.68% for control, 2.5% and 5% MPP, respectively, after 24 h incubation time. There was no significant increase (p > 0.05) in the probiotic log counts after 48 h incubation time for LF for all three concentrations of MPP taken. However, in the case of LGG, a statistically significant decrease (p < 0.05) was observed at 2.5% and 5% MPP concentrations after 24 hours of incubation. This indicates better viability of LF as compared to LGG at 48 h of incubation. The decrease in growth is attributed to the inability of LGG to utilize the nutrient substrate available and the accumulation of metabolic by-products, etc. Probiotic strain LF might have a different regime of carbohydrates utilizing enzymes present in the medium compared to LGG. An increase in MPP concentration from 2.5% to 5% also increased the log count of LF, whereas no statistically significant difference was observed in the case of LGG. In a similar study by [28], similar results were reported where a significant increase in log counts (1.25 log CFU/ml) of a probiotic strain L. casei in the MRS broth growth medium with 2% banana peel powder compared to control was observed after 24 h of incubation.

The effect of MPP on the growth of normal human gut enteric bacteria was determined using two different strains of enteric origin. Figures 4(c) and 4(d) represent the effects of different concentrations of MPP (0%, 2.5%, and 5%) supplemented in BHI broth as culture media on the growth of (C) Escherichia coli (EC) and (D) Enterococcus faecalis (EP) during 48 h incubation at 35 ± 2°C. The results depicted a significant (p < 0.05) increase in the number of both enteric bacteria after 24 h incubation. The log counts increased by 3.17, 1.673, and 1.128 log CFU/ml for EC and 3.928, 1.596, and 0.482 for EF at 0%, 2.5%, and 5% MPP concentration, respectively. The percentage increase was 44.76%, 23.21%, and 16.2% for EC and 45.35%, 17.904%, and
5.502% for EF for control, 2.5%, and 5% MPP, respectively, after 24 h incubation time. These values indicated that the MPP does not support the growth of enteric bacteria tested, and this effect increased with an increase in the concentration of MPP from 0 to 5%. Furthermore, after 48 h incubation, the increase in log CFU/ml for EC was 9.73% for control, whereas for 2.5% and 5% MPP, a decrease in log CFU/ml (3.67% and 4.89% respectively) was observed when compared to 24 h incubation time. Similar results were obtained by other researchers suggesting the selective stimulation of probiotic strains over enteric bacteria by tested prebiotic candidates. In one such study, the putative prebiotic potential of mango peel powder was reported by [27], wherein the capability to produce SCFAs by microbiota of human feces was assessed through in vitro colonic fermentation and revealed that the counts of Bifidobacterium spp. increased most abundantly amongst all the tested genera. Another study [61] also substantiated the fact of selective stimulation of probiotics over enteric commensals by prebiotics, where a cellulose-based dietary fiber (prebiotic) from banana peel has been found to selectively promote the growth of L. plantarum TISTR2075 over E. coli TISTR073. A recent study investigated the potential of mango peel powder (MPP) to stimulate the growth of probiotic strains and SCFA production after in vitro colonic fermentation. They found that MPP as such and yogurt incorporated MPP were able to maintain the counts of Bifdobacteria up to 8.11 ± 0.89 and 8.02 ± 1.01 log CFU/g, respectively, and had the highest SCFA production in both the cases as compared to other combinations. Results from this study suggested MPP as a functional ingredient with good prebiotic effects for food formulations designed for gut health. [55]

Maltodextrin (MD), a commercial prebiotic, was employed to compare the efficacy of MPP as a potential prebiotic candidate. Figures 5(a) and 5(b) represent the effects of different concentrations of MD (0%, 2.5%, and 5%) supplemented in MRS broth as culture media on the growth of (A) Lacticaseibacillus rhamnosus NCDC347 (LGG) and (B) Limosilactobacillus fermentum NCDC143 (LF) during

![Figure 4: Prebiotic effects of various concentrations of mango peel powder (MPP) on the number (log colony-forming units (CFU)/mL) of tested individual probiotic bacteria; (a) Lacticaseibacillus rhamnosus NCDC347; (b) Limosilactobacillus fermentum NCDC143; enteric bacterial strains (c) Escherichia coli ATCC25922; and (d) Enterococcus faecalis NCDC114 during 48 h of incubation at 35 ± 2°C. Different lowercase letters indicate a significant difference (p < 0.05) based on the concentration of MPP (0%, 2.5%, 5%) within the same incubation time points. Various uppercase letters indicate significant differences within the same concentrations at different incubation time points. Data are represented as mean values ± standard error (n = 3).](https://example.com/figure4.png)
48 h incubation at 35 ± 2°C. The results show a significant ($p < 0.05$) increase in the number of both probiotic strains tested. For LGG, the percentage increase in log CFU/ml for 2.5% and 5% MD concentrations was 57.75% and 59.85%, respectively, which were significantly lower as compared to that for 2.5% and 5% MPP (81.60% and 78.40%, respectively) after 24 hours of incubation. For LF, the percent log CFU/ml increase was 55.83% for 2.5% MD, which was comparable to that of 2.5% MPP (52.98%), whereas for 5% MD, the percentage increase (57.32%) was significantly lower than that of 5% MPP (93.68%). There was a significant decrease in log CFU/ml of both LGG and LF after 48 h incubation.

The overall growth pattern for probiotics and enteric bacterial strains was higher for MPP compared to MD. Similar results have been reported by Oliveira et al. [62], where the presence of maltodextrin increased the growth of probiotic strains of *L. bulgaricus*, *L. acidophilus B. lactis*, and *L. rhamnosus*. In another study by [63], the effect of probiotic strains with maltodextrin (as a placebo) was determined through metabarcoding. Probiotic strains, *L. rhamnosus* and *L. fermentum*, exhibited growth increments on maltodextrin and were retrieved in the probiotic-treated cohort as against other genera. Meanwhile, maltodextrin (placebo) had a positive impact on the abundance of bifidobacteria counts [63].

The effect of MD on the growth of enteric bacterial strains was also determined. Figures 5(c) and 5(d) represent the effects of different concentrations of MD (0%, 2.5%, and 5%) supplemented in BHI broth as culture media on the growth of *Escherichia coli ATCC 25922*, and *Enterococcus faecalis NCDC114* during 48 h of incubation at 35 ± 2°C. Different lowercase letters indicate a significant difference ($p < 0.05$) based on the concentration of maltodextrin (0%, 2.5%, and 5%) within the same incubation time points. Various uppercase letters indicate significant differences within the same concentrations at different incubation time points. Data are represented as mean values ± standard error ($n = 3$).
of MPP (44.76%, 23.21%, and 16.2%) for respective concentrations. In a previous study, maltodextrin was not as easily catabolized as glycogen and maltose by commensal *E. coli* K-12 and pathogenic strain *E. coli* O157:H7 in an *in vivo* experiment [64]. For EF, the percent increase was 63.76%, 96.5%, and 82.79%, as compared to 45.35%, 17.904%, and 5.502% for MPP for 0%, 2.5%, and 5% concentrations, respectively. These results suggest that MD supports relatively higher growth of selected enteric bacterial strains compared to MPP to be tested as a potential prebiotic candidate in this study. This indicates better performance of MPP as a prebiotic compared to MD.

Though *Enterococcus faecalis* is of enteric origin, several strains of this species have been appreciated as probiotics [65, 66]. In a study, researchers found that MD-based cryoprotectants were able to increase the growth rate of *Lactobacillus* spp. and *Enterococcus faecalis* [67]. Maltodextrin, in combination with other prebiotics, has been observed to stimulate the growth of probiotics over enteric bacterial stains selectively. For example, in a previous study, a prebiotic mixture (galacto-oligosaccharides + maltodextrins) was administered daily to human volunteers for up to 5 days. This prebiotic mixture increased the total fecal bifidobacterial count from 40.80% to 53.85% and simultaneously reduced the *E. coli* count from 55.35% to 45.06% [68].

3.8. Determination of Prebiotic Activity Scores. Data in Figure 6 and Table 3 depict the prebiotic scores of various concentrations of MPP and MD supplemented in respective culture media (MRS for probiotics; BHI for enteric bacteria) with different combinations of probiotic and enteric bacterial strains. It was evident that the prebiotic activity scores of MPP were significantly higher (*p* < 0.05) than those of MD for all the combinations, indicating that MPP was a better prebiotic compared to MD for the selected probiotic strains (Table 3). The prebiotic activity score of MPP was highest with LF v/s EF (5% MPP), followed by LF v/s EC (5% MPP), and lowest with LGG v/s EC (2.5%). Thus, as per the *in vitro* prebiotic activity score, MPP is a better prebiotic for LF than LGG. Similar findings on the mango peel powder as a potential prebiotic candidate have also been reported by [28]. The peels of various fruits, including mango peel, were also studied for their functional and prebiotic properties. Another finding by Sayago-Ayerdi et al. [27] also reported that predigested mango peel powder stimulated the growth of *Bifidobacterium* spp., the most compared to all other genera tested after 24 h in an *in vitro* human colonic fermentation assay.

In comparison, at 72 hours, the growth of *Bifidobacterium* and *Lactobacillus* spp. increased abundantly. The results obtained in this study clearly concluded that *Limosilactobacillus fermentum* NCDC143 has shown the best prebiotic score with *Langra* MPP among all other combinations tested. Several researchers have observed that dietary fibers from natural sources, specifically fruit peels, have shown better prebiotic potential than commercial ones [69–71]. In a similar study on assessing the prebiotic activity score of bergamot oligosaccharides (BOS) against fructo-oligosaccharide (FOS) over 24 h through a culture-independent microbial growth assay [72]. It was revealed that BOS had higher prebiotic index (PI) scores (6.90) compared to FOS (6.12) after 10 h incubation [72].

3.9. Effect of MPP on the Antioxidant Potential of the Probiotic Strains. A percentage DPPH inhibition assay was performed to determine the antioxidant potential of the probiotic strains using mango peel powder (MPP) as a substrate during 48 hours of incubation at 35 ± 2°C. Figures 7(a) and 7(b) depict the % DPPH inhibition activity of various concentrations of MPP (0%, 2.5%, and 5%) at different incubation time intervals (0, 24, and 48 h) for LGG and LF, respectively. Both LGG and LF strains showed a significant (*p* < 0.05) increase in the % DPPH inhibition activity with increasing concentration of MPP as well as incubation time. Before the start of fermentation, i.e., at 0 h, the % DPPH inhibition activity of LGG control (0% MPP) was 8.873%, which was much lower than that of 2.5% and 5% MPP.
(58.96% and 65.82% respectively). This suggests that MPP in itself has good antioxidant activity. As the fermentation proceeded, the antioxidant activity of LGG with 2.5% and 5% MPP increased significantly. Notably, the antioxidant activity of LGG with 0% MPP also increased significantly from 8.873% at 0 h to 45.605% at 24 h and 57.32% at 48 h, suggesting that LGG was able to produce metabolites with antioxidant potential without MPP. However, when supplemented with MPP, the % DPPH inhibition activity was significantly higher. A similar trend of increased antioxidant capacity with MPP was observed in the case of LF (Figure 7(b)).

At 0 h, the % DPPH inhibition activity of LF (control) was 9.807%, which increased significantly up to 57.92% and 63.21% at 24 h and 48 h, respectively, showing the capability of LF strain itself to produce more antioxidants. The addition of 2.5% and 5% MPP had shown a synergistic effect on the increase in the % DPPH activity (i.e., 70.54% and 77.55%, respectively) before fermentation. As the fermentation started, the antioxidant activity of LF with 2.5% and 5% MPP increased significantly from 80.93% to 87.03% at 24 and 48 hours, respectively, compared to the control (57.92%). MPP at 2.5% could increase the % DPPH inhibition activity significantly from 63.21% to 85.20%, suggesting the impact of the presence of MPP with LF strain for enhancing antioxidants in the medium. Nonsignificant differences were observed with a combination of 5% MPP + LF from 24 to 48 h in terms of increase in the % DPPH inhibition. Interestingly, out of the two probiotic strains tested, *Limosilactobacillus fermentum* NCDC143 had the higher overall % DPPH inhibition activity. A similar pattern of enhancement in the antioxidant potential of probiotic strains with mango, pomegranate, pineapple peels, or their extract has been reported [73–76]. For example, in a study, fermented soy milk exhibited better antioxidant activity (71.2 ± 4.0%) compared to control [73]. Likewise, more polyphenols, antioxidant activity, functional properties, and mineral bioavailability were observed in fermented pea nut press cakes [77–82], cereals, and pulses [83–89] compared to the control. A significant increase of 113% for DPPH-radical scavenging was reported after fermentation with lactic acid bacteria in a medium supplemented with 2% pomegranate peel extract [74]. Probiotic yogurt with pineapple peel possesses higher antioxidant capacity [76]. Such food products with bioactive compounds are helpful in prevention/cure of various disorders of the gastrointestinal tract. Recently, the bioactive compounds from mango peel have been appreciated for their antimicrobial, antioxidant, and anticancer activities [90]. Several studies have reported previously about the health-promoting properties of bioactive compounds such as polyphenols, flavonoids, tannins, etc. [91–94]. In a recent study, mangiferin (C-glucosylxanthone), a natural compound from mango, has been found to have a chemoprotective mechanism against colonic aberrant crypt foci (ACF) in rats by lowering ACF values and less colon tissue penetration induced by azoxymethane [86].

3.10. Extended Discussion/Limitations. The peel of *Mangifera indica* is a waste by-product from the juice industry, rich in antioxidants with around 40% dietary fiber on dry matter basis. In the present study, the chemical composition along with antioxidant capacity, structural parameters, and mean growth rate of probiotic strains in the presence of MPP and the probiotic activity score of mango peel powder at different concentrations were determined to evaluate its feasibility as a functional ingredient or natural prebiotic source in various food formulations. *In vitro* growth parameters of probiotics strains were highest at 24 h incubation which can be considered as the optimal time for fermentation. However, the predigestion of MPP in the simulated gut environment would give more authentic data of the
prebiotic score of Langra mango peel powder. Moreover, the production of SCFA, after passage through the simulated gut environment, is another essential attribute required to establish the fact that Langra MPP is an effective prebiotic source. Extraction of the pure form of dietary fiber such as pectic oligosaccharides or nonfiber components like polyphenols will be helpful in deducing the principal components or a combination, responsible for the selective stimulation of probiotic strains over Gram-negative gut pathogens or modulation of gut microbiota. Metagenomic profiling of major microbial residents of healthy human gut, after digestion of MPP or extracted pure form in an in vitro model of the human colon or in an in vivo experiment, is essentially needed in order to establish the prebiotic potential of Langra MPP.

4. Conclusions

By-products from fruits and vegetable processing possess immense potential to develop novel prebiotic or other functional food ingredients, besides maintaining a sustainable environment and new business opportunities. Langra mango peel powder (MPP) has several functional properties like high % DPPH inhibitory activity, total phenolic and flavonoid content, and good water and oil holding capacity, which makes it a desirable functional food additive. The present study primarily determined the prebiotic efficacy of MPP, which displayed a better prebiotic score compared to commercial prebiotic maltodextrin when fermented with Limosilactobacillus fermentum NCDC143 compared to L. rhamnosus GG and selectively stimulated their growth over enteric bacterial strains. Langra mango peel powder with Limosilactobacillus fermentum NCDC143 exhibited functionality to develop a synbiotic formulation based on the existing data, and further in vivo studies are warranted in this regard.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>GI</td>
<td>Geographical indication</td>
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<tr>
<td>BOD</td>
<td>Biological oxygen demand</td>
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<td>DF</td>
<td>Dietary fibers</td>
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<tr>
<td>PP</td>
<td>Polysaccharides</td>
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<tr>
<td>ATCC</td>
<td>American type culture collection</td>
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<tr>
<td>NDBI</td>
<td>National Dairy Research Institute</td>
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<tr>
<td>MPP</td>
<td>Mango peel powder</td>
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<tr>
<td>TDF</td>
<td>Total dietary fiber</td>
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<tr>
<td>DPPH</td>
<td>Diphenyl-2-picrylhydrazyl</td>
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<tr>
<td>TPC</td>
<td>Total phenolic content</td>
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<tr>
<td>GAE</td>
<td>Gallic acid equivalent</td>
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<tr>
<td>TFC</td>
<td>Total flavonoid content</td>
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<tr>
<td>QE</td>
<td>Quercetin equivalent</td>
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<tr>
<td>WHC</td>
<td>Water holding capacity</td>
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<tr>
<td>OHC</td>
<td>Oil holding capacity</td>
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<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
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<tr>
<td>TGA</td>
<td>Thermo-gravimetric analysis</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>MRS</td>
<td>De-Man Rogosa Sharpe</td>
</tr>
<tr>
<td>BHI</td>
<td>Brain heart infusion</td>
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<tr>
<td>MD</td>
<td>Maltodextrin</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<tr>
<td>EC</td>
<td>Escherichia coli</td>
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<tr>
<td>EF</td>
<td>Enterococcus faecalis</td>
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<tr>
<td>CFU</td>
<td>Colony-forming units</td>
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Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All the authors declare that they have no conflicts of interest.

Authors’ Contributions

KHJ performed experiments and conducted data compilation; PD performed experiments and conducted data compilation, writing, and editing; DCR performed reviewing and editing; CG conducted conceptualization, funding acquisition, performed experiments, and carried out data compilation, writing of the original draft, editing, and reviewing; BSS carried out writing, reviewing, and editing; JSD and SBD performed reviewing and editing; KHJ and CG have contributed equally to the article.

Acknowledgments

The authors acknowledge the funding from IOE Scheme/Seed Grant/Development scheme no. 6031/PFMS scheme no. 3254 provided by UGC, GoI, under the Institution of Eminence Scheme to Banaras Hindu University, Varanasi, India.

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