PREBIOTIC EFFECT, PHYTOCHEMICAL AND FUNCTIONAL COMPOUNDS FOUND IN SELECTED FRUIT PEELS: A NOVEL APPROACH TO SYNBIOTIC IN PHYTO-PROBIOTICS

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Abstract. Prebiotics have gained attention for their potential to modulate intestinal microbiota and promote gut health. This study explores the prebiotic potential of mango, banana, and olive fruit peels and their synbiotic relationship with probiotic strains. Fruit peels were analyzed for their composition, functional properties, and antioxidant potential. Results showed variations in moisture, ash, protein, fiber, fat, and carbohydrate content among the fruit peel powders. Total phenolic content and antioxidant activity showed significant differences. Functional groups and phytochemical compounds in the peel powders were identified by FTIR and GC-MS. The synbiotic relationship between the fruit peel powders and probiotic strains was investigated. Supplementation of the fruit peel powders significantly increased probiotic counts, indicating potential synbiotic effects. The proliferation of probiotic strains varied depending on the strain, peel powder concentration, and incubation time. Mango and banana peel powders showed promising effects on promoting probiotic proliferation, while olive peel powder generally exhibited less impact.
Keywords: *prebiotics, fruit peel powders, synbiotic relationship, phytochemical composition, functional properties, antioxidant activity*

Introduction

Prebiotics are non-digestible, short-chain carbohydrates (SCCs), which have gained significant attention to promote probiotics activity that normally resides in gastrointestinal tract (GIT). Non-digested carbohydrate molecules, including saccharides (di, oligo, and poly-), sugar polyols, and resistant starches possess prebiotic potential, and modulate the balance of intestinal microbiota, and thereby promote gut health (Wongkaew et al., 2022). Prebiotics promote a healthy gut environment by selectively stimulating the growth and activity of probiotics (Bamigbade et al., 2022). Prebiotics have been proved to exert potential physiological effects, which include improved gut barrier function, increased absorption of minerals, and enhanced immune system, while reducing the risk of chronic diseases, including cardiovascular disorders, obesity, and diabetes (Markowiak and Śliżewska, 2017). Commercially available food grade prebiotics are galactooligosaccharides (GOS), fructooligosaccharides (FOS), and inulin (Davani-Davari et al., 2019). GOS and FOS are derived from lactose and occur naturally in human milk. On the other hand, fructan and inulin are soluble dietary fibers (Bamigbade et al., 2022). These prebiotic compounds exhibit unique chemical

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structures, varying chain lengths or degrees of polymerization, and find diverse applications in the field of gut health. However, most prebiotics are derived from plant sources, and the use of these sources as prebiotics has not been widely explored. Prebiotics are found in various fruits, vegetables and crops, including bananas, onions, garlic, and wheat because they have the adequate quantity of oligofructose and inulin (Khan et al., 2023). In addition to proteins, vitamins, phenolic compounds, and minerals, fruit peels include substantial levels of fructooligosaccharides, pectin, and dietary fibers (Fierascu et al., 2020). Various prebiotics in plant wastes, including disaccharides, oligosaccharides, and non-digestible oligosaccharides, are found that are indigestible in the gut and subsequently fermented by lactic acid bacteria and bifidobacteria (You et al., 2022; Davani-Davari et al., 2019). Mango, banana, and olive fruit peels could also be the potential source of prebiotics, since they are rich in dietary fiber, fructooligosaccharides, and other valuable compounds that are not digestible by the human body (Zahid et al., 2021). The current study aims to evaluate the prebiotic potential of mango, olive, and banana fruit peels and to assess the synbiotic relationship between lactic acid bacterial strains isolated from these fruits.

Mango (*Mangifera indica*) is a tropical fruit that is widely consumed around the world, and its industrial products generate a significant amount (~50-55%) of waste in the form of seeds, peels, etc. Mangoes peels contain substantial number of dietary fibers that can potentially enhance the count of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus* (Wongkaew et al., 2022). Gut microbiota ferments dietary fiber, producing beneficial fatty acids (Fu et al., 2022). Studies have shown that mango pulp has potential prebiotic activities and can enhance the activity of starter in probiotic yogurts, increase the total bacterial counts, and improve the count of beneficial bacteria, such as lactobacilli bacteria and bifidobacteria (Hussein et al., 2020; Vicenssuto and De Castro, 2020; Zahid et al., 2021). Mangos peels are also enriched with nutrients and compounds that have nutraceutical potential, including antimicrobial, antioxidant, and anti-inflammatory activities (Lebaka et al., 2021; Kim et al., 2021; Maldonado-Celis et al., 2019; Hu et al., 2018). Additionally, polyphenols have potentially beneficial effects on gut microbes, as evidenced by their biotransformation into products that affect the proliferation of Bifidobacterium spp (Kumar Singh et al., 2019). Gut microbes utilize polyphenols found in mangoes to generate metabolites in significant amounts (Sáyago-Ayerdi et al., 2021, 2019; Bertha et al., 2019).

Bananas are another tropical fruit that is widely consumed, and their peels are also an excellent source of prebiotics. Banana peels contain fructooligosaccharides, which have Generally Recognized As Safe (GRAS) status and are widely used as prebiotics (Kurtoglu and Yildiz, 2011). Bananas contain approximately 60-80% indigestible carbohydrates such as starch, lignin, celluloses, and hemicelluloses, which have prebiotic characteristics (Powthong et al., 2020; Phillips et al., 2021). Studies have shown that oligosaccharides from banana peels can promote the growth of *Lactobacillus paracasei* and have strong prebiotic potential (Phirom-On and Apiraksakorn, 2021; Budhisatria et al., 2017; Cordoba et al., 2018).

Olives are a widely consumed fruit that is often used for their oil, but they also have potential health benefits due to their prebiotic content. Olive fruit and leaves have a variety of bioactive compounds, including phenolic compounds, which have prebiotic effects (Oliveira et al., 2021; Žugčić et al., 2019). Additionally, olive waste products, such as olive pomace, have been shown to have prebiotic potential due to their high fiber content (Ribeiro et al., 2021a, b).

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Probiotics are live microorganisms that confer health benefits when administered in adequate amounts. The use of synbiotics, which are a combination of probiotics and prebiotics, has been proposed as a potential strategy for improving gut health (Markowiak and Śliżewska, 2017). Lactic acid bacteria, such as Enterococcus and Lactobacillus, are commonly used as probiotics, and their synbiotic relationship with prebiotics has been investigated (Fijan, 2014). The aim of this study was to evaluate the prebiotic potential of olive, mango, and banana fruit peels and to assess the synbiotic relationship between lactic acid bacterial strains isolated from these fruits. In this study, we have evaluated the synbiotic relationship between lactic acid bacterial strains isolated from mango, banana, and olive, including *Enterococcus faecium* MW 585366 (Mango), *Enterococcus faecium* NBRC 100486 (Banana), and *Enterococcus durans* JCM 8725 (Olive).

Methodology

Probiotics cultures

The probiotics strains, *Enterococcus faecium* MW 585366, *Enterococcus durans* JCM 8725, *Enterococcus faecium* NBRC 100486, were obtained from National Institute for Genomics and Advanced Biotechnology (NIGAB) at National Agriculture Research Centre, Islamabad, Pakistan. The strains were sourced from the local fruits of Pakistan, including Mango (Chaunsa), Banana (Basrai), and Olive (Gemlik).

Preparation of fruit peel and pomace powder

Mango fruits (*Mangifera indica*) Chaunsa variety were procured from Mango Research Institute Multan, Pakistan. Meanwhile, the banana peels (*Musa cavendish 'Dwarf Cavendish*') and olive pomace (*Olea europea*) were obtained from Rashdi Agriculture Farm Khesana (Mori, Tando Allahyar, Sindh, Pakistan) and Barani Agriculture Research Institute (Chakwal, Punjab, Pakistan). The samples were dried at 60°C for 24 h in a hot air oven, and subsequently ground and sieved through a 0.25-mm sieve using a Microgrinder, following the method of Kamel et al. (2017).

Proximate composition of fruits peel powder

Air-dried Moisture percentage, Crude Proteins percentage, Crude Fat percentage, Crude fiber percentage, Total Ash percentage were calculated by using AOAC 2012 methods (AOAC, 2012). Total carbohydrates constitute were estimated by using following equation (Wongkaew et al., 2021; Ozabor et al., 2020):

$$\%\text{Carbohydrate} = 100 - (\%\text{ash} + \%\text{crude fat} + \%\text{crude fiber} + \%\text{crude protein} + \%\text{moisture content})$$

Antioxidant properties of MMP, BPP, and OPP

Preparation of MPP, BPP, and OPP extract through sonication method

The ultrasonic extraction procedure was conducted with slight modifications, following the method of Dauber et al. (2022). The extraction utilized 80% ethanol as a solvent in an ultrasonic bath (Model 300, Pulse, Italy), maintained at a constant frequency of 40 kHz and a temperature of 50 ± 5°C. The samples were sonicated for 60 min in the ultrasonic bath and subsequently filtered using Whatman filter papers (No. 4) under dark conditions. The filtered solutions were then centrifuged at 3000 rpm

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for 15 min using a top table centrifuge (BKC-TH 16RII). The extract produced was then stored in the dark for further analysis.

Determination of TPC

The Folin-Ciocalteu method, as described by Castro-Vargas et al. (2019), was used for the quantification of total phenolic (TPC) compounds in peels and pomace. Specifically, 100 µL (micro liter) of extract and 70 µL of Folin-Ciocalteu reagent (10% w/w) were mixed in a glass test tube, and allowed to incubate for 5 min, after which 750 µL Na_2CO_3 (Sodium carbonate) aqueous solution was added. After incubation in dark conditions for 90 min, the absorbance at 765 nm was determined using a BMV UV-1900 spectrophotometer. The triplicate test provided the results in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g).

DPPH (diphenyl-2- picrylhydrazyl) radical scavenging assay

The present study evaluated the antioxidant activity of extracts obtained from peels and pomace using the DPPH radical scavenging assay, with minor modifications as described by Kaur et al. (2021). 200 µL of the sample extracts were mixed with 1 mL of 100 mM methanolic DPPH solution, followed by shaking and incubation in the dark for 20 min. Controls without the sample were also prepared. The absorbance (A_S) of both samples and controls was measured at 517 nm, using a BMS UV-1900 spectrophotometer. Following equation was used to determine the scavenging activity of DPPH radical assay:

$$\text{Scavenging activity } \% = A_S \div A_0 \times 100$$

where A_S represents the sample's absorbance and A_0 represents the absorbance of control.

Water and oil holding capacity of FFP

The water holding capacity (WHC) and oil holding capacity (OHC) of the samples were determined using a modified version of the method developed by Salih et al. (2017). To measure WHC, 1 g of the sample was mixed with 30 mL of deionized water, vortexed for 1 min, and incubated at room temperature for 24 h. To measure OHC, the same procedure was followed using 30 mL of olive oil (Pak olive, NARC) instead of water, following the same procedure. After incubation, the samples were subjected to centrifugation at 3000 × g for 20 min (Hermle Germany, Model Z326K) and the supernatants were discarded. The residues were then weighed and compared with their initial weights to determine the amount of dry samples (1 gram) absorbed by deionized water or olive oil.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR is used to analyze essential structural information for various polymer types, including carbohydrate polymers. In this study, we employed an FTIR-8400 spectrophotometer (Shimadzu, Japan) to investigate the dried FPP. To prepare the samples for FTIR analysis, we utilized potassium bromide (KBr) and measured the absorbance within a range of 4000–400 cm^{-1}, as previously reported by Kaur and Gill (2021).

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GC-MS of fruits peel powders

Gas chromatography integrated with mass spectrometry (GC-MS) is utilized for the detection and identification of volatile compounds present in complex mixtures. In this study, we employed the GCMS-QP 5050A (Shimadzu, Japan), which incorporates a silica capillary column measuring 30 mm × 0.22 mm ID × 0.25 µm film. The carrier gas used was Helium at 1.8 mL per minute flow rate. The injector functioned at 220°C while the oven was initially set at 60°C for a duration of 2 min, after which it was raised at 240°C. The identification of components was based on Willey and National Institute of Standards and Technology (NIST) libraries as well as comparison of their retention indices. The chemical components were assessed quantitatively from the GC–17A with peak area of each compound and the results were displayed in the average after three consecutive experiments.

Our samples were prepared using HPLC grade methanol and a 1% dried extract of FPP. Subsequently, 1.0 µL of the FPP was injected into the equipment, and a GC cycle time of 36 min was used to obtain mass spectra of the FPP extract. These spectra were compared to those in the NIST mass-spectral libraries for the identification of compounds present in our sample.

Synbiotic assessment of FPP

For each analysis, the probiotic strains were grown in de Man, Rogosa and Sharpe (MRS) broth and incubated for 20-24 h for 37°C. The probiotic culture was then harvested at 4500 × g for 10 min at 4°C, and saline solution was used to wash the pellet thrice (Duarte et al., 2017). MRS broth was supplemented with various concentrations of fruit peel powders (FPP), including a control (0%, 2%, and 3% Inulin), and sterilized using a bench-top autoclave model (Hermle Germany, Model Z326K) at 121°C for 20 min. The study included nine FPP treatments: banana FPP (BPP 0%, BPP 2%, and BPP 4%), mango FPP (MPP 0%, MPP 2%, and MPP 4%), and olive pomace FPP (OPP 0%, OPP 2%, and OPP 4%). To determine colony-forming units per milliliter (CFU/mL) at different time intervals (1 h, 24 h, and 48 h), and the peptone water (0.1%) was used for ten-fold serial dilutions.

Statistical analysis of data

The proximate composition, antioxidant properties, and WHC and OHC were evaluated using one-way analysis of variance (ANOVA). To compare the means of the three treatments, Tukey's test was employed with a 95% confidence level. Minitab® 8.1 statistical software evaluated the effect of various combinations of the two independent variables (FPP and their concentrations) on the dependent variable (probiotic counts) through the application of the general linear mode (GLM).

Results

Proximate analysis of FPP

The proximate analysis evaluated the quality and potential functional characteristics of the dried fruit peel samples, while also providing their chemical composition. *Table 1* presents the findings of the proximate analysis of MPP, BPP, and OPP. The moisture contents of the dried FPPs were found to be significantly different. The moisture content

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varied between 7.26% and 11.0%. Specifically, the data revealed that BPP exhibited significantly highest moisture content (11.0%; $p < 0.05$), followed by MPP (8.40%) and OPP (7.26%). These variations in moisture content can influence the stability and shelf life of the fruit peel powders and may be attributed to their differing WHCs.

The ash content, which represents the residual inorganic material after complete combustion of organic combustion of organic components in a sample, varied among the three different fruit peel powders (FPP). The ash content ranged from 3.0% to 12.0%, with BPP having significantly higher ash content (12.0%; $p < .05$). However, no statistically significant difference was notable between the ash contents of MPP and OPP. Regarding protein content, OPP has the highest protein content at 8.4%, followed by BPP (7.41%) and MPP (3.33%). The fat contents of FPPs ranged from 2.70% to 22.73%. The OPP exhibited significantly higher crude fat contents (22.73%) compared to BPP (5.90%) and MPP (2.70%) due to its origin from olive oil residue ($p < 0.05$). The difference could be due to the inherent lipid content present in olive oil, resulting elevated fat levels in OPP.

Moreover, analysis of fiber content demonstrated that MPP exhibited significantly higher levels (35.16%) compared to BPP (12.00%) and OPP (5.03%). These variations suggest differences in fiber composition between MPP, BPP, and OPP. Carbohydrate content varied between 47.37% and 53.55% across the different FPPs. Among three different FPPS, MPP has the lowest carbohydrate content at 47.37%.

Table 1. *Proximate composition (%) of MPP, BPP, and OPP*

Fruit peels	Ash	Moisture	Crude fiber	Crude fat	Crude protein	Carbohydrate
MPP	3.0 ± 0.20^{b}	8.40 ± 0.20^{b}	35.17 ± 0.29^{a}	2.70 ± 0.20^{c}	3.33 ± 0.29^{c}	47.37 ± 0.15^{c}
BPP	12.0 ± 0.50^{a}	11.00 ± 0.50^{a}	12.00 ± 0.50^{b}	5.90 ± 0.10^{b}	7.42 ± 0.38^{b}	51.53 ± 0.15^{b}
OPP	3.0 ± 0.10^{b}	7.27 ± 0.15^{c}	5.03 ± 0.15^{c}	22.7 ± 0.208^{a}	8.42 ± 0.382^{a}	53.55 ± 0.377^{a}

BPP: Banana Peel Powder, MPP: Mango Peel Powder, OPP: Olive Peel Powder. The means ± standard deviation (n = 3) were calculated. Different superscript letters were used to indicate significant differences ($p < .05$) between means in the same column

Antioxidant properties of FPP

The three FPPs exhibited significant variations in their TPC and antioxidant potential (*Table 2*). Among them, OPP demonstrated the maximum TPC value of 38.67 mg GAE/d dw, whereas BPP had a lower TPC value of 24.03 mg GAE/d dw. The DPPH radical scavenging activity lies between 20.33%-61.05% across the three FPPs. In addition, MPP exhibited significantly higher DPPH radical scavenging activity (61.05%; $p < .05$). However, BPP demonstrated the lowest DPPH radical scavenging activity at 20.33%, while OPP had a DPPH value of 22.8%. Therefore, OPP exhibited the highest TPC value, while MPP demonstrated the most potent DPPH radical scavenging activity.

Water and oil holding capacity of FFPs

The present study demonstrated considerable variations in the functional characteristics of fruit peels (*Table 2*). The WHC results revealed that BPP exhibited the highest capacity (10.16%), while OPP and MPP have 3.66% and 3.6% WHC, respectively. Similarly, OPP displayed the highest oil holding capacity (2.88%),

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followed by BPP (2.07%) and MPP (2.54%). These results indicate that different fruit peels possess varying levels of water and oil-holding capacities, signifying their potential as natural sources of bioactive components.

Table 2. *Antioxidant activities and functional characteristics of MPP, BPP, and OPP*

Fruit peels	TPC	DPPH scavenging assay	WHC	OHC
MPP	31.73 ± 0.45^{b}	61.5 ± 0.17^{a}	3.6 ± 0.12^{c}	2.54 ± 0.2^{b}
BPP	24.03 ± 0.85^{c}	20.33 ± 0.29^{c}	10.16 ± 0.2^{a}	2.07 ± 0.12^{c}
OPP	38.67 ± 0.41^{a}	22.83 ± 0.06^{b}	3.66 ± 1.39^{b}	2.88 ± 0.25^{a}

BPP: Banana Peel Powder, DPPH: Diphenyl-2- Picrylhydrazyl, dw: dry weight, mg AAE/g: milligrams of ascorbic acid equivalents per gram, mg GAE/g: milligrams of gallic acid equivalents per gram, MPP: Mango Peel Powder, OHC: Oil Holding Capacity, OPP: Olive Peel Powder. TPC: Total Phenolic Content. WHC: Water Holding Capacity. The means ± standard deviation (n = 3) were calculated. Different superscript letters were used to indicate significant differences ($p < .05$) between means in the same column

Phytochemical screening of FPPs

FT-IR

FTIR analysis identified the functional groups present in the FPPs by analyzing the peak values in the IR spectra. The MPP, BPP, and OPP were subjected to FTIR analysis, which allowed for the separation of functional groups based on their characteristic peaks (*Fig. 1*).

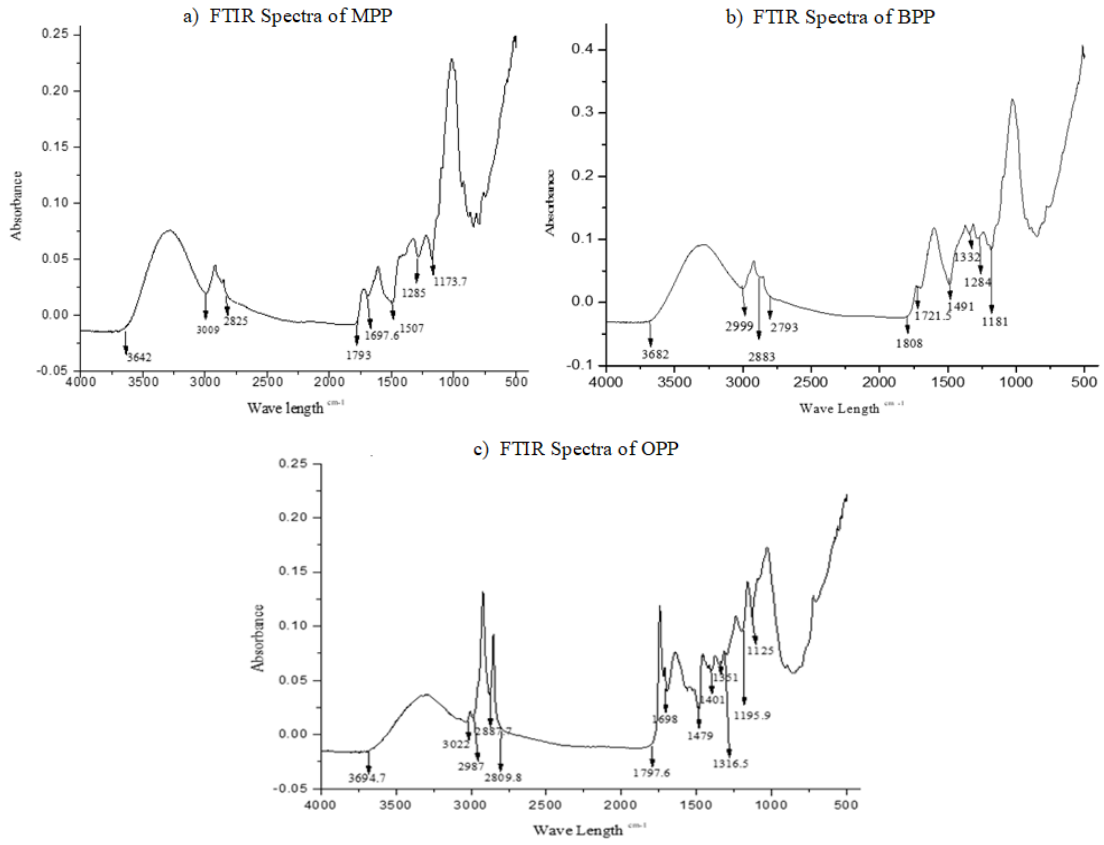

Figure 1. *FTIR spectra of (a) mango, (b) banana, and (c) olive peel powder*

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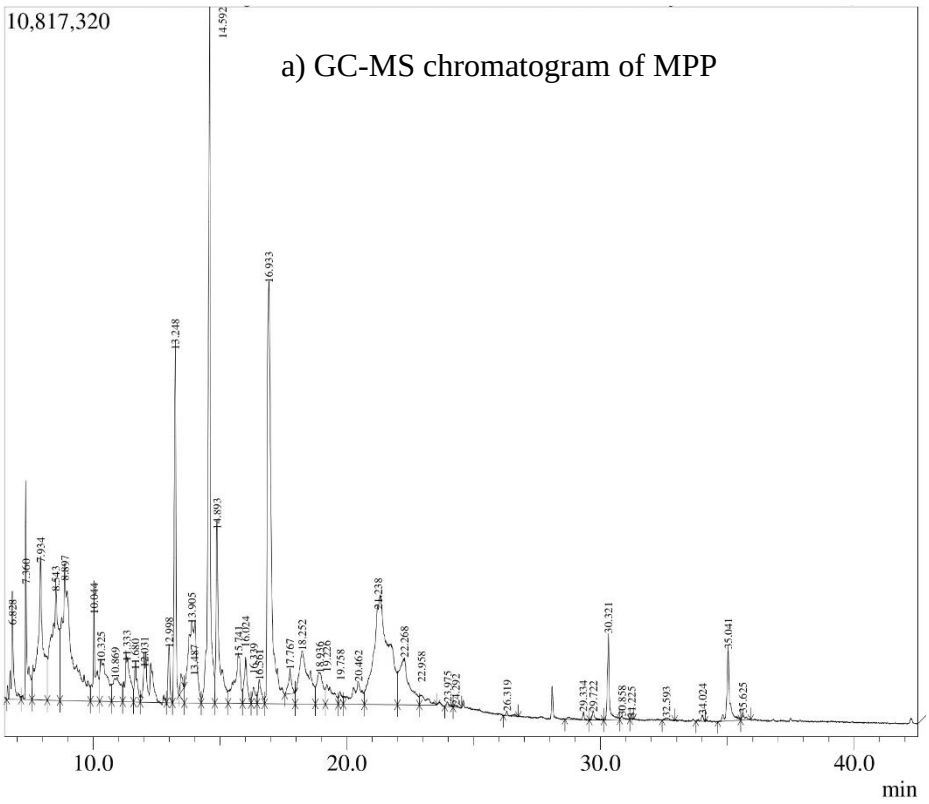
The FTIR analysis of the dried peel powders was conducted within the absorbance range of 450-4500 cm^{-1}. The obtained results revealed the presence of various functional groups in the samples, including alcohol, alkane, alkene, ester, amine, nitrile, phenol, aliphatic compounds, aromatic compounds, thiols, and sulfides. The presence of phenols, aliphatic arrangements, and hydroxyl groups (O-H stretching vibration) were indicated by prominent peaks in the FTIR spectra of MPP. Additionally, C-H stretching bands were detected, suggesting the presence of alkane groups. Other functional groups identified in MPP include C = O carbonyl groups, C-N stretching, C-S-C functional groups, and C-O ester groups. For BPP, the FTIR peaks exhibited an O-H free bond, thiol functional group and C-H stretching evocates vibration of –CH3 groups. Furthermore, C = O carbonyl group and C = O stretching as a cyclic imide were observed. Asymmetric and symmetric carboxylic acid groups, –CH2 bonds, and C-O stretch of esters were also identified in the FTIR spectra of BPP. In the case of OPP, the FTIR analysis revealed peaks corresponding to the O-H group, asymmetric and symmetric C-H groups, symmetrical –CH3 methyl stretching, C = O (carbonyl group), C-O in the carboxylic functional group, and N-H stretching. These results indicate various functional groups in OPP, including amine groups, carboxylic acid, and hydroxyl group. The identification of these functional groups in MPP, BPP, and OPP provides valuable insights into the diverse chemical composition of three examined FPPs, contributing to the overall characteristics and properties of MPP, BPP, and OPP.

GCMS

The phytochemical compounds present in the dried peel powder of mango, banana, and olive were analyzed by GC-MS (*Fig. 2*).



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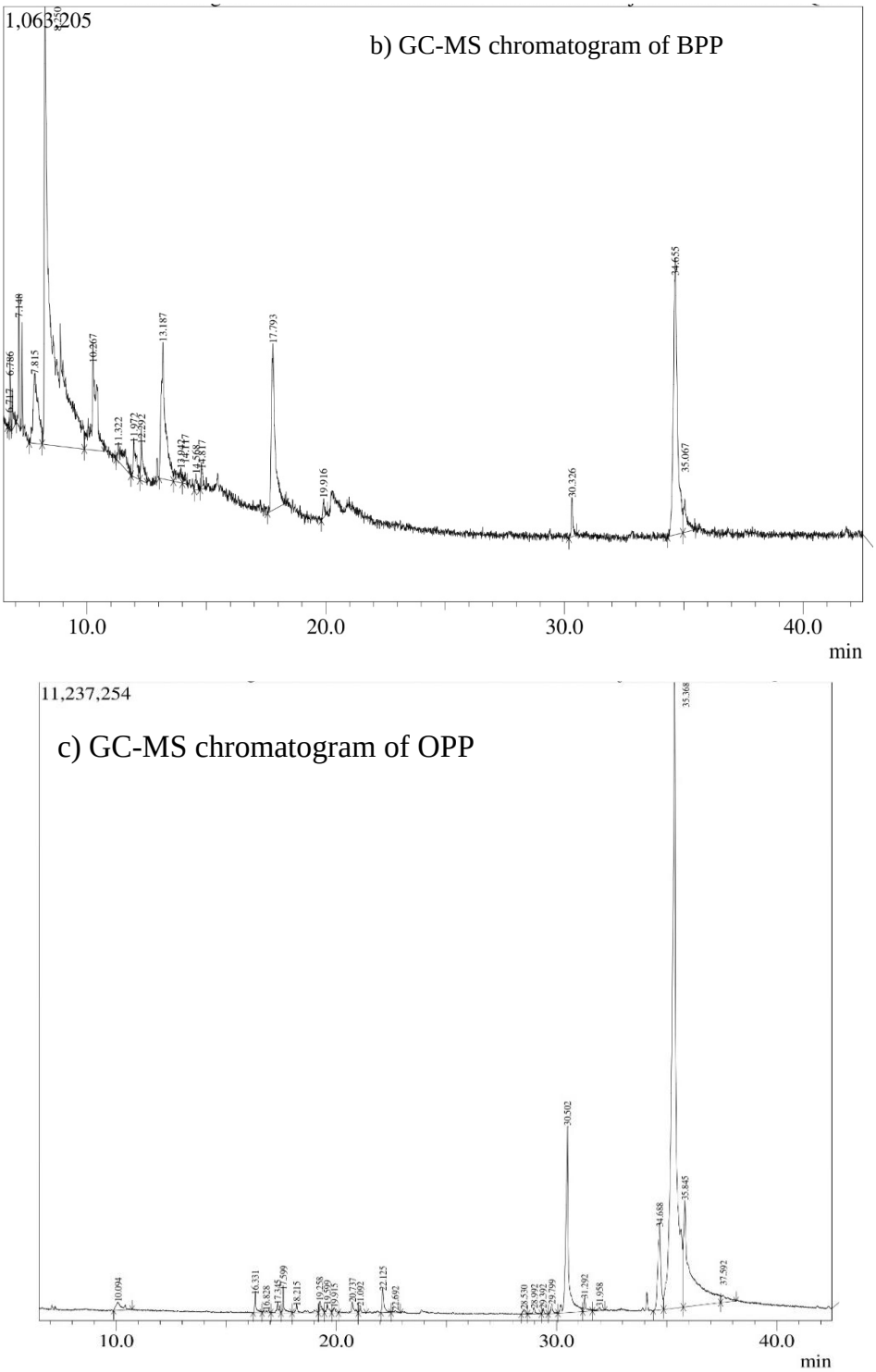

Figure 2. *GC-MS chromatogram of (a) mango, (b) banana, and (c) olive peel powder*

Some of the notable phytochemicals identified in the peel extract include Butanoic acid, 2-ethyl-3-oxo-, methyl ester (5.06%), DL-Arabinose (5.38%), Dihydroxyacetone (DHA) (8.79%), 5-Hydroxymethylfurfural (10.32%), 1,2,3-Benzenetriol (12.14%), and 1-Heptanol (11.12%). It is noteworthy that Butanoic acid, 2-ethyl-3-oxo-, methyl ester has not been previously reported in the literature as being present in any fruit skins or

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peels. Notable compounds include Dihydroxyacetone (DHA) (43.33%), 2-Hydroxy-gamma-butyrolactone (6.93%), DL-Arabinose (8.02%), Sucrose (8.80%), and 1,2-Benzenedicarboxylic acid, dioctyl ester (BADE) (15.72%). Dihydroxyacetone (DHA) is identified as a triose sugar. Some significant compounds include n-Hexadecanoic acid (11.06), 1,2-Benzenedicarboxylic acid (5.53), Oleic Acid (57.02), and Ethyl Oleate (16.55).

Synbiotic effects of FPPs

In the current study, synbiotic relationship and its effects on the growth of selected probiotic strains, *Enterococcus durans* JCM8725, *Enterococcus faecium* MW585366, and *Enterococcus faecium* NBRC 100486 was evaluated. The effects of varying concentrations (0%, 2%, and 4%) of FPPs (MPP, BPP, and OPP) were examined under different aerobic incubation times (1 h, 24 h, and 48 h) (*Table 3*).

Table 3. *Effects of different peel powders on Probiotic proliferation at varying concentrations and incubation times*

Probiotic strain	Concentration (%)	Incubation time (h)	Supplement			
			MPP	BPP	OPP	Inulin
Enterococcus faecium MW585366	0%	1	6.85 ± 0.05^{lm}	6.85 ± 0.05^{lm}	6.85 ± 0.05^{lm}	6.85 ± 0.05^{lm}
	2%	1	8.24 ± 0.23^{fghij}	8.24 ± 0.23^{fghij}	8.24 ± 0.23^{fghij}	8.24 ± 0.23^{fghij}
	4%	1	8.14 ± 0.17^{ghijk}	8.14 ± 0.17^{ghijk}	8.14 ± 0.17^{ghijk}	8.14 ± 0.17^{ghijk}
	0%	24	7.96 ± 0.04^{hijk}	7.79 ± 0.15^{jk}	7.03 ± 0.15^{lm}	7.50 ± 0.25^{kl}
	2%	24	9.47 ± 0.42^{a}	9.08 ± 0.51^{cd}	8.55 ± 0.38^{cd}	8.80 ± 0.26^{cdefg}
	4%	24	8.90 ± 0.36^{cdef}	8.87 ± 0.09^{cdef}	8.33 ± 0.28^{efghij}	9.18 ± 0.18^{abc}
	0%	48	6.77 ± 0.24^{m}	7.90 ± 0.08^{ijk}	7.05 ± 0.13^{lm}	7.52 ± 0.18^{kl}
	2%	48	8.65 ± 0.16^{c}	8.67 ± 0.10^{cdefg}	9.36 ± 0.14^{ab}	8.90 ± 0.05^{cdef}
	4%	48	8.40 ± 0.06^{defghi}	9.07 ± 0.10^{cd}	8.94 ± 0.25^{abcde}	8.88 ± 0.13^{cdef}
p-Value	0.000					
Enterococcus durans JCM8725	0%	1	7.39 ± 0.16^{kl}	7.39 ± 0.16^{k}	7.39 ± 0.16^{kl}	7.39 ± 0.16
	2%	1	8.03 ± 0.15^{fghijk}	7.22 ± 0.23^{l}	7.49 ± 0.05^{kl}	7.97 ± 0.06^{ghijk}
	4%	1	7.56 ± 0.04^{jkl}	8.23 ± 0.21^{efghij}	7.59 ± 0.10^{ijkl}	7.87 ± 0.10^{hijkl}
	0%	24	8.97 ± 0.44^{bcd}	8.57 ± 0.10^{hi}	8.97 ± 0.44^{bcd}	8.97 ± 0.44^{bcd}
	2%	24	9.02 ± 0.13^{bc}	9.06 ± 0.16^{bc}	8.93 ± 0.08^{bcde}	9.37 ± 0.13^{ab}
	4%	24	8.57 ± 0.10^{c}	8.96 ± 0.12^{defg}	9.79 ± 0.07^{a}	9.28 ± 0.30^{ab}
	0%	48	9.36 ± 0.33^{ab}	8.75 ± 0.05^{bcde}	8.27 ± 0.23^{defghi}	8.27 ± 0.23^{defghi}
	2%	48	8.71 ± 0.10^{bcdef}	9.00 ± 0.15^{bc}	8.67 ± 0.08^{bcdefg}	8.88 ± 0.08^{bcde}
	4%	48	8.75 ± 0.05^{bcde}	8.76 ± 0.15^{bcde}	9.36 ± 0.38^{bc}	9.03 ± 0.16^{bc}
p-Value	0.000					
Enterococcus faecium NBRC 100486	0%	1	7.71 ± 0.32^{ef}	7.71 ± 0.32^{ef}	7.71 ± 0.32^{ef}	7.71 ± 0.32^{ef}
	2%	1	7.57 ± 0.10^{f}	6.52 ± 0.07^{g}	7.62 ± 0.25^{f}	7.58 ± 0.19^{f}
	4%	1	7.51 ± 0.11^{f}	7.39 ± 0.14^{fg}	7.60 ± 0.17^{f}	7.73 ± 0.21^{def}
	0%	24	8.97 ± 0.44^{abc}	8.88 ± 0.08^{abc}	8.97 ± 0.44^{abc}	8.55 ± 0.41^{bcde}
	2%	24	9.06 ± 0.17^{ab}	8.85 ± 0.05^{abc}	9.05 ± 0.06^{ab}	8.97 ± 0.06^{abc}
	4%	24	8.84 ± 0.16^{abc}	9.51 ± 0.44^{a}	9.24 ± 0.35^{ab}	9.05 ± 0.18^{ab}
	0%	48	8.97 ± 0.11^{abc}	8.57 ± 0.41^{bcde}	8.57 ± 0.41^{bcde}	8.57 ± 0.41^{bcde}
	2%	48	8.89 ± 0.08^{abc}	8.95 ± 0.04^{abc}	8.61 ± 0.34^{bcd}	9.05 ± 0.13^{ab}
	4%	48	8.13 ± 0.21^{cdef}	9.06 ± 0.25^{ab}	8.94 ± 0.12^{abc}	8.98 ± 0.10^{abc}
p-Value	0.000					

BPP: Banana Peel Powder, MPP: Mango Peel Powder, OPP: Olive Peel Powder. The means ± standard deviation (n = 9) log CFU/ml were calculated. Different superscript letters were used to indicate significant differences ($p < .05$) between means in the same column

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The supplementation of prebiotics significantly increases ($p < .05$) probiotic count (log CFU/ml). The significant increase observed in the probiotics provide strong evidence supporting the role of the supplemented FPPs as a prebiotic agent that effectively promotes the proliferation of the diverse array of tested probiotic strains, i.e., *Enterococcus durans* JCM8725, *Enterococcus faecium* MW585366, and *Enterococcus faecium* NBRC 100486.

Among the probiotic strains studied, *Enterococcus durans* JCM8725 exhibited the highest increase in count (0.83 log CFU/ml) when supplemented with MPP after 24 h of incubation time. In contrast, the increase in BPP and OPP concentration and incubation time did not lead to a considerable increase in probiotic counts. Similarly, *Enterococcus faecium* MW585366 displayed an increase in count up to 0.59 log CFU/ml when supplemented with 2% concentration of MPP. However, when commercial prebiotic inulin was added at a 4% concentration, the count increased by 0.89 log CFU/ml after 24 h of incubation. Moreover, *Enterococcus faecium* NBRC 100486 was also subjected to supplementation with different concentrations of OPP, BPP, and inulin. This resulted in an increase in count of 1.02 log CFU/ml and 1.37 log CFU/ml at 2% and 4% concentrations, respectively, after 24 h of incubation.

Discussion

The present study comprehensively investigated the phytochemical constituents and the synbiotic relationship of prebiotics, including *Enterococcus faecium* MW 585366 (Mango), *Enterococcus faecium* NBRC 100486 (Banana), and *Enterococcus durans* JCM 8725 (Olive), isolated from mango, banana, and olive, respectively. The chemical composition analysis was performed using FTIR and GCMS, while the functional properties were assessed through proximate analysis and antioxidant properties. To examine the synbiotic effects, various concentrations (0%, 2%, and 4%) of selected FPPs were added to each probiotic strain. All the three tested FPP concentrations significantly ($p < .05$) increases viable probiotic counts after 24 h of incubation, indicating a potential synbiotic relationship between the fruit peel powders and the probiotic strains.

Understanding the composition of FPP, specifically the protein, fiber, and carbohydrate contents, is crucial for assessing their nutritional value and potential utilization in various food industries. The protein, fiber, and carbohydrate contents of three FPPs (OPP, BPP, and MPP) were examined. Proximate analysis was conducted following the AOAC methods for moisture, ash, protein, fat, and carbohydrate content (AOAC, 2012). The current study reported significantly higher moisture content of the BPP at 11.0% ($p < .05$), compared to MPP (8.40%) and OPP (7.26%). In line to this, previous studies by Kabenge et al. (2018) and Ahmed et al. (2021) reported moisture contents of 11.56% and 10.03% in BPP, similar to Romelle et al. (2016) (10.44%). The moisture content of the USA variety was found to be significantly higher at 22.06%, while the India variety had a lower moisture content of 9.52% (Puraikalan, 2018). Similar higher moisture values ranging from 9.52% to 13.5% were reported previously (Kabenge et al., 2018; Okoye et al., 2020; Roa et al., 2021; Chaudhary et al., 2022; Abubakar et al., 2016) for different BPPs. Furthermore, the moisture content of MPP in our study was higher than previous findings, i.e., 7.784% (Kaur and Srivastav, 2018) and 7.57% (Tahir et al., 2021). For olive, the highest and lowest moisture content values reported were 10% (Dinc and Yel, 2018) and 2.12% (Inzunza-Soto et al., 2021),

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respectively, which varied depending on the extraction method. In addition to moisture content, this study also found significantly higher ash content of banana peel powder (12%; $p < .05$). These findings align with the previous studies, as Puraikalan (2018) reported similar value of ash content (12.9%) in banana peel. However, Zahid et al. (2021) reported highest ash content of 16.07% in BPP. Regarding the MPP and OPP, our results showed no significant difference, which is correlated with the previous studies showing similar trend (Dukare et al., 2022). Ash content generally represents the inorganic residue left behind after complete combustion of organic matter and contains minerals, including calcium, magnesium, potassium, and trace elements. The observed differences in ash content between BPP and the other peel powders could be due to variations in the mineral composition of the fruit peels. The differences in ash content of different peel powders might be due to various factors, such as climatic conditions, banana varieties, ripening stages, etc. (Romelle et al., 2016). The significantly higher ash level in banana peel powder suggests the presence of valuable compounds, indicating its potential as a natural source of calcium, zinc, and iron (Mohd Zaini et al., 2022). Banana peels are known to contain relatively higher amounts of potassium compared to other fruit peels (Hussein et al., 2019). This variation in mineral composition could explain the lack of significant difference in ash content between BPP, MPP, and OPP. Incorporating banana peel powder into certain diets may help augment the intake of these essential minerals. Furthermore, the different varieties of olive have been reported to exhibit variations in ash contents ranging from 11.25% to 16.68% (Nunes et al., 2021). Although this specific variation is not directly related to the present study, it demonstrates the diversity in ash content among different fruit varieties. The protein contents of the three FPP were determined, with OPP (8.41%) exhibiting significantly highest protein content compared to BPP (7.41%) and MPP (3.33%). Previously, Foti et al. (2022) reported protein content in olive pomace about 2.6/100 g, while 6.64% OPP protein was also reported previously (Cravotto et al., 2022). FPPs with higher protein content can significantly increases the nutrient density of the food products. By incorporating these powders into food formulations, the overall nutritional profile can be enhanced, providing a greater range of essential amino acids and promoting a balanced diet (Marçal and Pintado, 2021; Gupta et al., 2023). In addition to protein contents, OPP also have significantly higher fat content (22.73%), which was higher from previously reported fat contents, i.e., 11.72% (Inzunza-Soto et al., 2021) and 15% (Sinrod et al., 2019). Moreover, the fiber content analysis revealed that MPP exhibited the highest fiber content (35.16%), which was similar to the findings of (Zahid et al., 2021), with fiber content of 47.01% in MPP. The abundance of dietary fiber in MPP holds significant potential for enhancing the quality and nutritional value of food products, including bakery applications (Salehi and Aghajanzadeh, 2020). Among the three FPP, MPP exhibited the lowest carbohydrate content at 50.15%. However, Romelle et al. (2016) and Kaur and Srivastav (2018) reported highest carbohydrate content of 63.80% and 66.4% in MPP, respectively. These findings highlight the considerable variation in carbohydrate content among FPP and emphasize the need for careful selection and characterization of fruit peels to optimize their moisture, protein, ash, fiber, fat, and carbohydrate composition. Furthermore, the chemical contents in FPP can be influenced by the extraction method employed, the choice of solvents, and the specific fruit varieties utilized (Cravotto et al., 2022). These findings suggest that careful selection of extraction methods and solvents, as well as consideration of fruit varieties, are essential in optimizing the protein content of FPP.

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Antioxidant potential and TPC of three FPPs subjected to hot air oven drying were examined. Significant differences were observed among the FPPs, with OPP displaying the maximum TPC value of 38.67 mg GAE/g dw, higher than BPP (TPC: 24.03 mg GAE/g dw). A study conducted on Spain's olives demonstrated a TPC value of 17.67 mg GAE/g dw (Quero et al., 2022). Similarly, previous studies reported a varying TPC value of 19.71 mg GAE/g dw (Goldsmith et al., 2018), and 3.1 mg GAE/g dw (Tapia-Quirós et al., 2020) for OPP. For MPP, this study found a TPC value of 31.73 mg GAE/g dw, which is similar to 27.51 mg GAE/g dw (Suleria et al., 2020), lower than 67.58 mg GAE/g dw (Safdar et al., 2017), and 54.2 mg GAE/g dw (Lanjekar et al., 2022), but higher than 9 mg GAE/g dw (Guandalini et al., 2019). Previous studies reported the lowest TPC value of 0.55 mg GAE/g dw (Ramírez Damián et al., 2022) and 1.21 mg GAE/g dw (Zahid et al., 2021) for BPP. In addition, 6.13 mg GAE/g dw TPC value was also reported for BPP (Suleria et al., 2020). However, as compared to previous literature, we observed significantly higher TPC value of 24.03 mg GAE/g dw for BPP. Extraction conditions, solvents used, and different methods could influence the TPC of fruit peels.

In the present study, MPP demonstrated higher DPPH assay (61.5%; $p < .05$) compared to other FPP samples, while BPP exhibited lowest scavenging activity (20.33%). In addition, Dukare et al. (2022) also reported a similar DPPH value of 59.9% in mango peels dried at 80°C. This result is in line with previous studies reporting DPPH values of 70% for Ataulfo mango (Rojas et al., 2015) and 79.6% for Indian mango (Ajila et al., 2010). However, our study found lower DPPH radical scavenging activity of MPP, compared to a previous study reporting 90%-92% (Kaur et al., 2022; Ordoñez-Torres et al., 2021). In addition, Madalageri et al. (2015) reported the highest 96.18% DPPH value for mango peel. Previous investigations comprehensively found antioxidant compounds within mango peel extracts, including isohamnetin, kaempferol 3-glucoside, myricetin, quercetin 3-glucoside, quercetin piranoside, and rutin (Souza et al., 2019). The discovery and assessment of these phenolic compounds add to our expanding understanding of the bioactive constituents found in MPP. This highlights the value of agro waste and byproducts for extracting valuable phenolic compounds that can be applied in diverse applications, including pharmaceuticals, nutraceuticals, and functional foods.

The water and oil-holding capacity of fruit peels are crucial factors in various industrial applications. The WHC is an important parameter that indicates the ability of these materials to retain water. In this study, there was significant differences in the WHC and OHC among the three FPPs. The WHC of BPP was significantly higher (10.16%; $p < .05$) compared to MPP (3.6%) and OPP (3.66%). However, the WHC of BPP (10.16%) in this study is higher than previous reports, in which WHC ranged from 1.02 to 7.30 g water/g sample (Salih et al., 2017). Zahid et al. (2021) also revealed that the WHC of BPP was 5.94 g water/g peel powder. In addition, the WHC of banana peel fiber was significantly higher than that of banana fiber, with values of 2.80 g/g and 2.66 g/g, respectively (Mohd Dom et al., 2021). This suggests that banana peel fiber has a greater capacity to hold water compared to banana fiber. Additionally, the WHC and OHC of banana peel flour were within the range of 5.8-6.4 g/g and 1.8-2.3 g/g, respectively (Bakar et al., 2018). These values indicate the ability of banana peel flour to retain water and oil. The observed differences in WHC values between different studies could attributes toward various factors. The method of analysis, including differences in the experimental conditions such as temperature, pH, and ionic strength,

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can affect the hydration properties of materials (Lucarini et al., 2020). Furthermore, the chemical structure, particle size, and composition of the materials, including the presence of starch, dietary fiber, and protein, can also effects WHC (Waliullah et al., 2020). Similarly, the protein and fiber content of BPP was higher, thereby contributing to the higher WHC of BPP in the present study. Moreover, OHC capacity of OPP (2.88%; $p < .05$) was significantly higher than MPP (2.54%) and BPP (2.07%). The OHC of BPP found in this study align with the results of Bakar et al. (2018), i.e., 1.8-2.3 g oil/g sample of OHC in banana peel flour. The OHC values of three examined FPPs were lower than WHC, suggesting that FPPs have a greater capability to absorb and retain water molecules compared to oil molecules. However, the WHC and OHC of MPP were lower and higher, respectively, compared to 6.4 g/g WHC and 1.6 g/g OHC in mango fiber concentrate (Siew Lian and Chong, 2015). The observed variations in the WHC and OHC can be attributed to the dissimilarities in the chemical composition and structural properties of the fruit peels. The higher WHC values may be attributed to the lower loss of soluble proteins and the presence of higher levels of polar amino acids within the fruit peel powders. On the other hand, the lower OHC values can be attributed to the hydrophobic nature of proteins and the binding performance of non-polar amino acid side chains to fat molecules (Meng et al., 2023).

FTIR spectroscopy was utilized to analyze the functional groups present in the mango, banana, and orange peel powders (MPP, BPP, and OPP). The FTIR analysis revealed the presence of various functional groups in the samples, including hydroxyl groups, phenols, alkanes, esters, amines, nitriles, and sulfides. Specific peaks in the FTIR spectra indicated the presence of these functional groups, providing insights into the chemical composition of the peel powders. For instance, the high abundance of hydroxyl groups in banana peel is consistent with previous studies (Akter et al., 2021). In addition, GC-MS analysis was performed for comprehensive identification and quantification of phytochemicals present among all three FPPs. The results revealed a diverse array of phytochemicals present in these fruit peel powders, each with potential bioactive properties. MPP exhibited the presence of 43 phytochemicals, some of the identified compounds, such as butanoic acid, 2-ethyl-3-oxo-, methyl ester, and 1,2,3-benzenetriol. DL-Arabinose, another compound detected in MPP, is a monosaccharide widely used in the in vitro culture of various microorganisms (Cruz-Moreno et al., 2023). Dihydroxyacetone (DHA), classified as a triose sugar, was also found in MPP. Interestingly, DHA serves as a precursor to antimicrobial compounds present in Leptosermum spp. honey (Obeng-Darko et al., 2022). Additionally, 5-hydroxymethylfurfural, identified as a member of aldehydes and a secondary metabolite, has been studied for its potential biological activities (Zulfina et al., 2021). The analysis of BPP demonstrated the presence of 19 phytochemicals, one of them is 2-hydroxy-gamma-butyrolactone, which has been reported for its insecticidal properties (Bharali et al., 2017). DL-Arabinosei is also identified in BPP. Additionally, sucrose, a well-known disaccharide, can serve as a carbon source (Anichebe et al., 2019). The analysis of OPP revealed 24 phytochemical compounds. Among these, n-hexadecanoic acid, commonly known as palmitic acid, was found to possess antioxidant, anticancer, and anti-inflammatory properties, along with potential antibacterial effects against biofilm-forming bacteria (Syed et al., 2022). 1,2-benzenedicarboxylic acid, considered a promising phenolic bioactive compound, exhibited antioxidant, antimicrobial, and anti-fouling activities (Banni and Jayaraj, 2023; Yarazari and Jayaraj, 2022; Kumar and Goel, 2019). Oleic acid, classified as a monounsaturated fatty acid (MUFA), has been

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associated with various nutraceutical benefits, including cardiovascular disease prevention, diabetes management, anti-hypertensive effects, and anti-inflammatory properties (Chen et al., 2022). Another fatty acid, ethyl oleate, was identified in OPP, and its presence in olive mill wastewater has been reported. Kuley et al. (2017) demonstrated the inhibitory effects of olive mill wastewater, which contains ethyl oleate, on fish spoilage bacteria. One of the significant findings of this study is the identification of a novel compound, butanoic acid, 2-ethyl-3-oxo-, methyl ester, in the fruit peel powders. To the best of our knowledge, there is no existing literature reporting the presence of this compound in any fruit skins and peels. This discovery adds to the growing body of knowledge regarding the phytochemical composition of fruit peels and highlights the unique chemical profile of these plant-derived materials. Further investigation is required to explore the potential bioactive properties and potential applications of this novel compound in various fields, such as pharmaceutical, nutraceutical, and food industries.

Furthermore, this study investigated the synbiotic relationship between FPP and probiotic strains. Results showed that prebiotic supplementation led to a significant increase in probiotic count. *Enterococcus durans* JCM8725 showed the highest increase in count when supplemented with 2% MPP and 4% BPP for 24 h. 4% BPP also significantly promoted higher proliferation rate for *Enterococcus faecium* NBRC 100486.

For *Enterococcus faecium* NBRC 100486, both MPP and OPP at 2% concentration had the same count of 8.55 log CFU/ml, which is the lowest among the peel powders tested. The highest count is observed with Inulin (9.6 log CFU/ml), followed by BPP (9.25 log CFU/ml). BPP seems to promote the greatest proliferation of *Enterococcus faecium* NBRC 100486. At 2% concentration, all FPPs showed similar counts of *Enterococcus faecium* NBRC 100486 (9.05 log CFU/ml), while at 4% concentration, the highest count is observed with 4% Inulin (9.5). No significant difference was found in promoting proliferation compared to the control (Inulin). The trends observed in 24-h incubation may not necessarily be the same for 48-h incubation. MPP and BPP show promising effects on promoting probiotic proliferation, while Inulin consistently exhibits a positive influence across different strains and conditions. OPP generally shows lower proliferation compared to other peel powders in the tested conditions.

The combination of prebiotics and probiotics has shown promise in promoting gut health and overall well-being. Investigating the potential symbiotic effects of FPP on the viability and growth of selected probiotic strains could contribute to the development of functional foods with enhanced nutritional and health benefits.

Conclusion

This study investigated the phytochemical composition and synbiotic relationship of fruit peel powder (FPP) derived from mango, banana, and olives. The FPPs showed variations in moisture, ash, protein, fiber, fat, and carbohydrate content. MPP had higher protein and fiber content, while BPP had higher moisture and ash content. OPP had the highest fat content. FTIR and GCMS analysis provided insights into the functional groups and phytochemical constituents. The synbiotic relationship between FPPs and probiotic strains was investigated, with supplementation significantly increasing probiotic counts. The proliferation strains varied depending on the strain, FPP concentration, and incubation time. 2% concentration of MPP and BPP showed

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promising effects in promoting probiotic proliferation, while inulin consistently exhibited a positive influence across different strains and conditions. OPP generally showed lower proliferation compared to other peel powders. Future research should focus on elucidating bioactive compounds, investigating the mechanism of action behind the synbiotic relationship, and optimizing extraction methods and processing techniques. Further studies are needed to determine the therapeutic applications of FPPs, including modulating gut health, preventing chronic diseases, and improving overall well-being.

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Conflict of interests. The authors declare no competing interest.

Novelty statement. The fruit peels are obtained from different local varieties of the fruits of Pakistan. Likewise, the strains which are used in the study of synbiotic relationship were obtained from non-dairy, and non-fermented food sources. These strains were obtained from fresh fruits. It is a novel approach that the lactic acid producing strains isolated from non-dairy and non-fermented foods were used as probiotics with prebiotics in synbiotic relationship. The synbiotic relationship between the Mango, Banana and Olive peel powders and probiotic strains was investigated. Mango and banana peel powders showed promising effects on promoting probiotic proliferation, while olive peel powder generally exhibited less impact.

Author contributions. Fasiha Fayyaz Khan, Asma Sohail, Shakira Ghazanfar, and Aayesha Riaz were involved in the study conception and design. Material preparation, data collection, and analysis were performed by Fasiha Fayyaz Khan, Asma Sohail, and Asif Ahmad. The first draft of the manuscript was written by Fasiha Fayyaz Khan, and all authors provided feedback on previous versions and approved the final manuscript.

Data availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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