

Evaluation of Prebiotic and Antioxidant Potential of Citrus Peel Waste

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Abstract

Background: In Punjab, citrus processing industries generate 40 million tones of citrus waste each year. Citrus fruit peels have been studied with reference to antioxidant potential and dietary fiber only. There are insufficient studies in context to the prebiotic potential of citrus fruit peels. Prebiotic potential of the fruit peels may provide an option to develop functional foods with a cost effective ingredient.

Materials and Methods: Citrus fruits such as kinnow (with or without albedo), lemon, and orange (with or without albedo) were purchased. Peels were separated manually and processed for further analysis. Bioactive compounds and antioxidant activity were analyzed using standard method. The Fourier-transform infrared spectroscopy was used to analyze prebiotic oligosaccharides and prebiotic potential of peels was also assessed.

Results: The study concluded that the maximum total phenolic and flavonoid content was found in Orange without albedo (14.07 mg GAE/g) and (10.02 mg QE/g), respectively. Orange without albedo exhibited the highest antioxidant activity i.e., 82.97 percent RSA whereas orange with albedo showed maximum FRAP activity which was 17.32 mg FeSO₄. The highest amount of total dietary fiber content was found in orange with albedo peel powder with an average value of 51.99 percent. However, kinnow peel without albedo showed the highest value for probiotic viability i.e., 7.61 CFU/ml.

Conclusion: Kinnow peel without albedo with highest prebiotic potential may be used to develop prebiotic rich products.

Keywords: Citrus Peel; Prebiotic Oligosaccharide; Antioxidant Potential

Abbreviations

µg: Micro Gram; mg: Milligram; g: Gram; kg: kilogram; mm: Millimetre; psi: Pounds Per Square Inch; °C: Degree Celsius; FPP: Fruit Peel Powder; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; TDF: Total Dietary Fiber; IDF: Insoluble Dietary Fiber; SDF: Soluble Dietary Fiber; FTIR: Fourier Transform Infrared; AOAC: Association of Official Analytical Chemists; AACC: American Association for Clinical Chemistry; QE: Quercetin Equivalent; GAE: Gallic Acid Equivalent; pH: Potential of Hydrogen; CFU: Colony Forming Unit

Introduction

A large amount of fruit by-products such as pomace, seeds, peel, and spoiled fruits are produced during fresh consumption as well as food processing. These fruit by-products specifically peel are excellent sources of bioactive components and prebiotic oligosaccharides [1]. The Food and Agricultural Organization (FAO) has given some interesting figures of by-products based on the total production of fruits per year. One of the fruit processing industries which deals in orange processing produces 50 percent of bagasse [2]. With an estimated 88 million tonnes of production annually, just

one-third of citrus fruits are processed and make up the biggest category of fruit crops in the world. 98 percent of industrialised crops fall into this category, which includes oranges, grapefruit, lemons, and mandarins. Citrus industry residues are made up of peel, pulp, and seeds. And, peels have been widely used as a functional food ingredient in numerous commercial formulations, such as bakery and confectionary products, as a source of antioxidants [3]. In Punjab, citrus processing industries generate 40 million tonnes of citrus waste each year [4]. In general, citrus fruit peels have been studied with reference to antioxidant potential and dietary fiber only. And, there are insufficient studies in context to the prebiotic potential of citrus fruit peels.

Prebiotics are specifically fermented carbohydrates that alter the composition and activity of the gut microbiota and promote the host's health [5]. In general, Dietary fiber (DF) has been associated with prebiotic activities. However, all the DFs do not show evidence of prebiotic activity [6]. There are basic three principles to consider an ingredient as a prebiotic. Firstly, a considered prebiotic must have resistance to gastrointestinal absorption, secondly, it should be fermentable by gut microbes and thirdly, it must have properties to selectively improve the probiotic effect of bacteria linked with health benefits [5]. And, oligosaccharides such as pectic oligosaccharides (POS), fructooligosaccharides (FOS), galactooligosaccharides (GOS), and xylooligosaccharides (XOS) have all these properties to be considered as the most important prebiotics. These are termed as bifidogenic substances due to their selective response to the growth of *Bifidobacterium* species [7].

Prebiotics has enormous potential for modifying the gut microbiota and these may be used as an alternative to probiotics or as additional support for them. Gut microbiota is a constantly changing ecosystem that contains trillions of bacteria and is influenced by a variety of factors such as dietary habits, seasonality, lifestyle, stress, antibiotic use, and diseases [8]. So, there is a need to pro-

vide prebiotic sources to gut microbes on daily basis to keep them healthy. Growing health awareness among consumers in present times has motivated food industries to seek out for cost-effective DF ingredient having unique properties such as prebiotics that allow attaining novel properties in health products. Therefore, the present study has been planned to evaluate the prebiotic and antioxidant potential of citrus peel waste for their intended use in product formulation.

Materials and Methods

Procurement of fruits

Kinnow, orange, and lemon were procured from the local market of Ludhiana. Kinnow's skin was smooth with no deep creases, firm to somewhat soft, and deep orange to almost crimson in color. Orange was rough with deep pores, firm with thick flesh, and light orange to deep orange color. Lemon had a medium-sized, thin peel, smooth skin, and a green to yellowish color.

Preparation of fruit peel powder

Fruits were cleaned and washed with tap water. Then, peels were separated from whole fruit manually with knives after being rinsed in tap water. Fruit peels were then cut into little pieces with sharp knives. Three types of fruit peel powders (FPPs) were made of each fruit peel: peel powder without any treatment, peel powder after blanching, and peel powder after autoclaving (Figure 1). The peel samples from orange and kinnow were also taken with and without albedo for the preparation of powder. Blanching was done for 2-3 minutes at 100 °C in hot water. After that, blanched peels were cooled down immediately in the ice bath. Then, samples were dried in a tray dryer at 55 °C till constant weight. Dried samples were cooled down and ground in an electric grinder. In the second treatment, samples were autoclaved at 121 psi for 30 minutes. After that, steps similar to the first treatment were followed. The prepared peel powders were stored in airtight glass bottles in the refrigerator until further analysis.

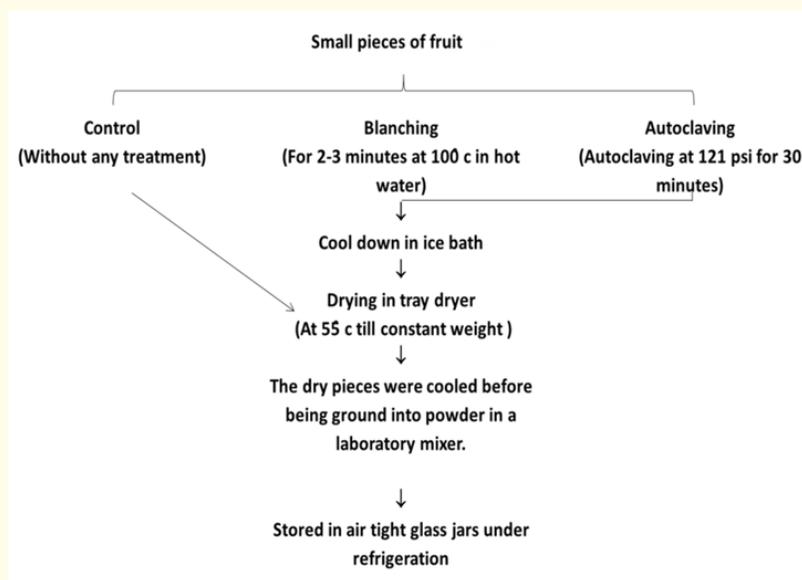


Figure 1: Flow diagram for sample preparation of FPP.

Bioactive compounds present in fruit peels

Total phenols. A sample of 0.5ml of methanolic extract was taken in a test tube and the volume was made up to 1 ml by adding 0.5 ml of 80 percent methanol. A 5 ml of Folin-Ciocalteu reagent was then put into the test tube and shaken thoroughly. After 5 minutes, 4 ml of a saturated solution of sodium carbonate was added. After incubation for two hours, absorbance was measured at 765 nm to estimate total phenols [9].

Flavonoids. A sample of 2 ml of methanolic extract of the sample was taken in a test tube and subjected to analyze flavonoid content [10]. A 0.1 ml of 1M potassium acetate, 0.1 ml of 10 percent aluminium chloride, and 2.8 ml of distilled water were added to the test tubes. The incubation of the mixer was carried out at room temperature for 30 minutes. The absorbance of the mixture was observed at 415 nm.

Antioxidant activity of fruit peels

Antioxidant activity of the samples was carried out by two methods viz., 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) [11] and Ferric Reducing Antioxidant Power (FRAP) [12] assays. For the DPPH assay, 2.9 ml of DPPH solution was added to 0.1 ml of methanolic extract. The mixture was incubated for 30 minutes in dark. The absorbance was measured at 517 nm for DPPH discoloration.

The DPPH scavenging effect was determined by the following formula.

$$\% \text{ inhibition} = \frac{A_B - A_A}{A_B} \times 100$$

Where, A_B = absorbance of blank, A_A = absorbance of sample

For FRAP assay, a sample of 0.3 ml was taken in a test tube and a volume made up to 1 ml with distilled water. A 1.8 ml of freshly prepared FRAP working reagent was put into each test tube and the content was incubated at 37°C for 10 minutes. The blue color complex was read at 593 nm against blank.

Characterization of prebiotic oligosaccharides

The characterization of prebiotic oligosaccharides of FPP with consideration of control peel powder (PP), blanched PP and autoclaved PP was carried out using Fourier transform infrared spectrophotometer (FTIR) (Alpha Bruker, USA). The sample of FPP which was kept on the FTIR sample holder and infrared spectra were noticed at wavelengths ranging between 400 and 4,000 cm⁻¹

Dietary fiber

A total dietary fiber assay kit (TDF 100 A kit) of sigma-Aldrich was used to estimate the insoluble and soluble dietary fiber with an enzymatic and gravimetric method. Total dietary fiber is a combination of both insoluble dietary fiber (IDF) and soluble dietary

fiber (SDF). Peel powders were defatted as per the kit module. A 50 ml of 6.0 pH of phosphate buffer was added to 1 gram of each sample. After, α amylase (0.10ml) with product code A 3306, protease (50mg/ml) with product code P 3910, and amyloglucosidase (0.1 ml) with product codes A 9913 were added step by step in samples. At every step, samples were kept for the water bath, and incubation and adjustment of pH were done according to the kit module. Precipitation of insoluble and soluble dietary fiber was done with 95 percent of ethanol. Filtration of precipitated dietary fiber was done with grade 1 sintered crucibles. A filtered mass of insoluble and soluble dietary fiber was dried in the oven. Further, protein and ash analysis was done.

Prebiotic potential of fruit peels

The prebiotic potential was evaluated by the standard method [13]. The probiotic strain i.e., *Lactobacillus plantarum* was inoculated into de Man Rogosa and Sharpe (MRS) broth (Figure 2). *Lactobacillus plantarum* was added to MRS broth together with various fruit peel powders at a concentration of 0.1 percent, and the mixture was then incubated for 48 hours at 37 °C. The viability of the probiotic was assessed during cold storage at 0, 7, 14, 21, and 28 days for these samples, which were kept at 4 °C (Figure 3 and 4).

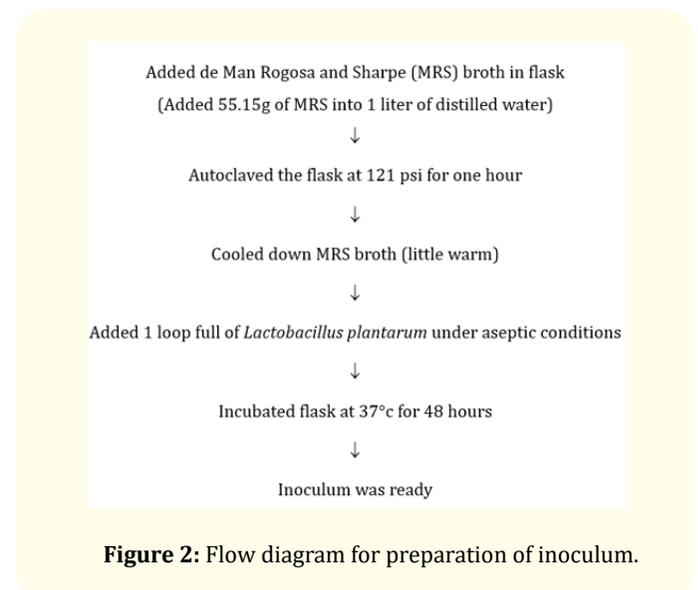


Figure 2: Flow diagram for preparation of inoculum.

Statistical analysis

All the determinations were carried out in triplicate and the results are given in mean \pm standard deviation. One-way and two-way analysis of variance (ANOVA) was applied for the statistical analysis of data using data SPSS 26 (statistical package for the social sciences) and SAS (Statistical Analysis System). To measure the difference between different treatments Tukey's test ($p < 0.05$) was performed and superscripts were added to present the differences.

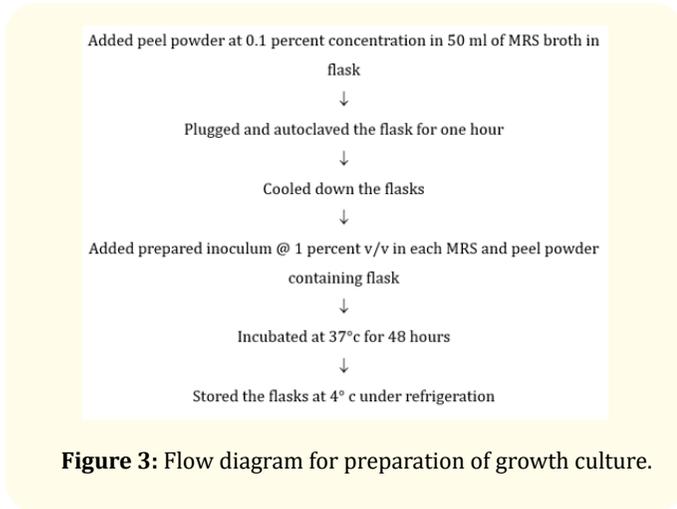


Figure 3: Flow diagram for preparation of growth culture.

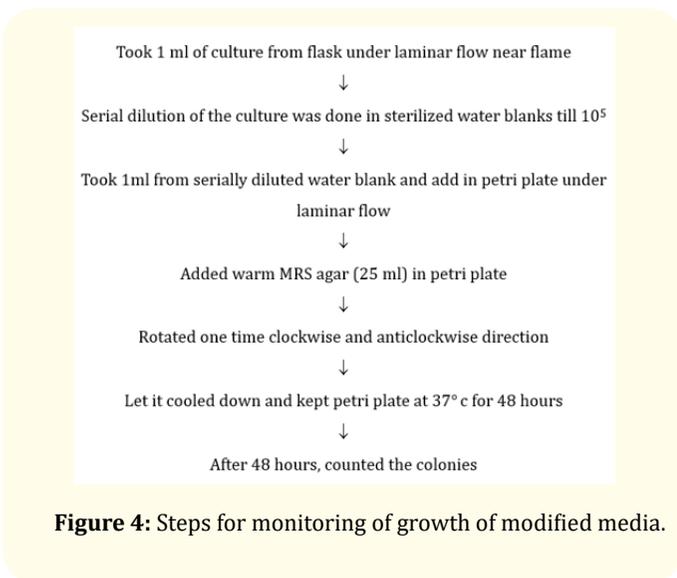


Figure 4: Steps for monitoring of growth of modified media.

Results and Discussion
Bioactive Compounds

The main contributor to the anti-oxidant properties of dietary products is phenolic acids. The results of the phenolic content of control, blanched and autoclaved fruit peel powders are presented in table 1. Statistically significant (p < 0.05) differences were observed within peels and treatments, along with control. Minimum phenolic content was observed in the autoclaved lemon peel powder (7.62 mg GAE/1g). However, kinnow without albedo control had 12.06 mg GAE/1g phenols which decreased significantly (p < 0.05) with treatments, and this trend was also seen among all peel powders. This might be due to the fact of leaching in water and exposure of peel to heat which can cause lower the content of phenolic with treatment. Maximum phenolic content was observed in the control orange (14.07 mg GAE/1g) and after blanching, phenolic content decreased significantly (p < 0.05) from 14.07 to 6.30mg GAE/1g. Thus, a huge loss of phenols was seen after blanching, this could be due to the fact that fruit peels were exposed to heat and pressure for a longer period which loosened cell wall tissues and caused the leaching of phenols. Previous studies also reported

that blanching caused leaching of phenols into water [14,15]. One study reported that total phenolic content of apple varieties such as cortland, golden delicious and beauty was 103.2, 97.7, and 93.0 mg GAE/g, respectively [16]. Thus, it showed that phenolic content depends upon the variety and the origin of the fruit. Blanching has a different effect on different vegetable; some show increased phenolic content and other shows decreased phenolic content [17]. In addition, researchers revealed that when there was an increment in total phenolic content, the reason behind this was the removal of tannins with the help of heat which enhanced the availability of phenolic content [18].

Type of peel powder	Phenols (mg GAE/1g)			Flavonoids (mg QE/1g)		
	Control	Blanched	Auto-claved	Control	Blanched	Auto-claved
Kinnow (With albedo)	10.88 _{xB} ± 0.1	10.44 _{xyB} ± 0.25	10.24 _{yB} ± 0.28	6.33 _{zC} ± 0.07	9.17 _{xA} ± 0.19	7.99 _y ± 0.35
Kinnow (Without albedo)	12.06 _{xB} ± 0.41	10.52 _{yB} ± 0.26	10.50 _{yAB} ± 0.01	9.45 _{xB} ± 0.16	9.75 _{xA} ± 0.35	9.59 _{xA} ± 0.17
Lemon	08.15 _{xD} ± 0.24	08.61 _{xC} ± 0.52	07.62 _{xC} ± 0.45	8.42 _{xB} ± 0.18	6.32 _{yB} ± 0.09	8.52 _{xA} ± 0.41
Orange (With albedo)	11.84 _{xB} ± 0.20	11.33 _{xA} ± 0.80	11.29 _{xA} ± 0.34	4.25 _{zD} ± 0.17	6.62 _{yB} ± 0.37	9.81 _{xA} ± 0.31
Orange (Without albedo)	14.07 _{xA} ± 0.03	06.30 _{yD} ± 0.33	06.94 _{yD} ± 0.31	10.02 _{xA} ± 0.20	9.45 _{xyA} ± 0.35	8.75 _{yAB} ± 0.44

Table 1: Content of bioactive compounds present in different fruit peels

Values represent mean ± SD, (n = 3), Values having different superscripts from x, y, z are significantly (p < 0.05) different from each other among treatments, Values having different superscripts from A, B, C to E are significantly different from each other day wise in a row.

Flavonoids, which are naturally occurring antioxidants found in food, are colored plant pigments that are mostly made from phenylalanine. The total flavonoid content of different fruit peels is presented in table 1. Through analysis, it was seen that Kinnow without albedo had 10.02, 9.45, and 8.75 mg QE/1g in control, blanched, and autoclaved, respectively. In the control fruit peel powder, the least flavonoid content was observed in orange with albedo 4.25 mg QE/1g and it was increased significantly (p ≤ 0.05) from 4.25 to 6.62 mg QE/1g with heat treatment in the blanched sample and in autoclaved, again it significantly (p ≤ 0.05) increased 9.81 mg QE/1g. Thus, the overall same trend was followed by citrus peels, in which flavonoid content increased after blanching except for lemon and orange with albedo. Furthermore, it was observed that flavonoid content decreased in autoclaving treatment in contrast to blanched samples. This might be due to the leaching of fla-

vonoids into the water during autoclaving. Moreover, the oxidation of flavonoids can also occur with heat treatments (19). Recently, it was also documented that even within the same class of flavonoids, different types of flavonoids respond differently to the same cooking procedure [20].

Antioxidant activity

Antioxidants convert DPPH, a stable free radical that accepts a hydrogen electron to produce a stable diamagnetic molecule, to a yellow compound. The results of DPPH radical scavenging activity (RSA) for control, blanched and autoclaved fruit peel powder are presented in table 2. The results revealed that after blanching and autoclaving DPPH radical scavenging activity significantly ($p < 0.05$) decreased in comparison to control samples. The maximum RSA was seen in orange without albedo peel powder as, in control, blanched and autoclaved the RSA was 82.97 percent, 81.33 percent, and 82.71 percent respectively. In kinnow without albedo, the control sample had shown 57.79 percent of DPPH radical scavenging activity and it decreased significantly ($p < 0.05$) from 57.79 to 44.87 percent in blanched fruit peel powder. Similarly, DPPH activity also decreased significantly ($p < 0.05$) in autoclaved samples from 44.87 to 39.23 percent. The striking feature of the trend is that there was an overall decrease in RSA after blanching and autoclaving in comparison to the control sample and this might be due to fact of leaching of various bioactive compounds. The leaching of bioactive compounds into the water might be due to the loose structure of the compounds with heat treatment [21]. Moreover, heat also causes the thermal degradation of bioactive compounds which reduces their amount after cooking [22].

Further, the results of FRAP value for control, blanched, and autoclaved different fruit peel powder are given in Table 2. It was found that Ferric reducing antioxidant power increased significantly ($p \leq 0.05$) in some fruit peels during blanching and autoclaving in comparison to the control. In citrus peel waste, there was almost the same FRAP value found in all samples among treatments and control fruit peel powder. It was evident that kinnow without albedo had 17.07 mg FeSO₄ equivalent/1 g in the control sample and significantly ($p \leq 0.05$) decreased after treatments. The main cause of the fall of FRAP value in both treatments might be due to the leaching of phenolic compounds [23]. Researchers also found that pomegranate peel had a maximum FRAP value followed by other fruit peels [24].

FTIR characterization of prebiotic oligosaccharides

Chemical bonds in molecules were identified using FTIR. The fundamental chemical and physical state of the material was revealed by FTIR. The chemical fingerprint of the sample is its spectra profile, which is used to screen and scan a wide range of components. It is a very useful technique for identifying functional groupings that are present in the sample [25]. The range of wavenumber was between 400-4000 cm⁻¹ and there were two regions; first was the fingerprint group region which was below 1500 cm⁻¹ and the second was the functional group region which was above 1500 cm⁻¹. Further, there were bond regions viz., 2500-4000 cm⁻¹ (single bond region), 2000-2500 cm⁻¹ (triple bond region), 1500-2000 cm⁻¹ (double bond region), and 600-1500 cm⁻¹ (fingerprint region) (Figure 5).

Type of peel powder	DPPH (%)			FRAP (mg FeSO ₄ equivalent/1g)		
	Control	Blanched	Auto-claved	Control	Blanched	Auto-claved
Kinnow (With albedo)	45.28 _x ± 6.39	36.87 _x ± 1.42	35.28 _x ± 2.58	16.25 _x ± 0.04	16.31 _x ± 0.08	16.22 _x ± 0.16
Kinnow (Without albedo)	57.79 _x ± 4.30	44.87 _y ± 2.38	39.23 _b ± 1.62	17.07 _x ± 0.10	16.88 _x ± 0.71	16.39 _y ± 0.12
Lemon	15.64 _x ± 1.54	14.15 _x ± 1.07	09.58 _y ± 2.79	17.16 _x ± 0.14	14.00 _y ± 0.02	16.56 _x ± 0.65
Orange (With albedo)	48.05 _x ± 4.23	28.25 _y ± 3.02	20.00 _z ± 0.30	17.32 _{xy} ± 0.00	17.50 _x ± 0.00	16.90 _y ± 0.39
Orange (Without albedo)	82.97 _x ± 0.08	81.33 _y ± 0.08	82.71 _x ± 0.54	16.26 _x ± 0.04	17.05 _x ± 0.46	15.66 _x ± 1.36

Table 2: Antioxidant activity present in different fruit peels.

Values represent mean ± SD, (n = 3), Values having different superscripts from x, y, z are significantly ($p < 0.05$) different from each other among treatments, Values having different superscripts from A, B, C to E are significantly different from each other day wise in rows.



Figure 5

Total Dietary Fiber

The present study showed the effect of blanched and autoclaved on the IDF content of different fruit peel powders. The trend shows that there was a significant ($p < 0.05$) increase in content from control to blanch to autoclaved fruit peel powder (Table 3). The maximum content of IDF was seen in orange with albedo i.e., 49.1, 49.22 percent, and 51.22 percent in control blanched and autoclaved

FPPs, respectively. Whereas kinnow without albedo control had 38.89 percent of IDF, the blanched sample had 39.41 percent and autoclaved had 40.78 percent of IDF which means the percentage of IDF increased with treatments which might be due to fact of the breakdown of cell walls or retrograde starch synthesis caused by heat or dryness during processing were the causes of the rise in IDF [26].

Fruit peel	Insoluble Dietary fiber			Soluble Dietary fiber			Total Dietary fiber		
	Control	Blanched	Autoclaved	Control	Blanched	Autoclaved	Control	Blanched	Autoclaved
Kinnow (With albedo)	44.50 ± 0.80 ^x	45.99 ± 0.99 ^x	46.39 ± 0.86 ^x	5.50 ± 0.11 ^x	5.67 ± 0.12 ^x	5.80 ± 0.17 ^x	50.30 ± 0.82 ^x	51.66 ± 1.11 ^x	51.89 ± 0.88 ^x
Kinnow (Without albedo)	38.89 ± 1.68 ^x	39.41 ± 0 ^x	40.78 ± 1.61 ^x	4.39 ± 0.02 ^x	4.45 ± 0.12 ^x	4.50 ± 0.03 ^x	43.39 ± 1.70 ^x	43.86 ± 0.12 ^x	45.17 ± 1.60 ^x
Lemon	38.02 ± 0.34 ^y	38.46 ± 0.53 ^y	40.55 ± 0.40 ^x	3.92 ± 0.11 ^z	4.56 ± 0.07 ^y	4.98 ± 0.20 ^x	43.00 ± 0.47 ^y	43.02 ± 0.53 ^y	44.47 ± 0.30 ^x
Orange (With albedo)	49.1 ± 1.94 ^x	49.22 ± 0.22 ^x	51.22 ± 1.56 ^x	2.52 ± 0.02 ^z	2.60 ± 0.03 ^y	2.89 ± 0.03 ^x	51.99 ± 1.92 ^x	51.82 ± 0.25 ^x	53.74 ± 1.58 ^x
Orange (Without albedo)	44.96 ± 1.86 ^x	45.23 ± 0.36 ^x	45.59 ± 0.90 ^x	1.80 ± 0.07 ^y	1.89 ± 0.00 ^y	2.23 ± 0.09 ^{xj}	47.19 ± 1.87 ^x	47.12 ± 0.35 ^x	47.39 ± 0.86 ^x

Table 3: IDF, SDF, and TDF (%) present in different fruit peels.

Values represent mean ± SD, (n = 3), Values having different superscripts from x, y, z are significantly ($p < 0.05$) different from each other among treatments, Values having different superscripts from A, B, C to E are significantly different from each other day wise in rows.

IDF: Insoluble Dietary Fiber, SDF: Soluble Dietary Fiber; TDF: Total Dietary

There was an increase in the SDF after blanching and autoclaving (Table 3) and this trend was followed by all citrus peel waste. The lowest SDF content among all peels was found in orange FPP (without albedo) in all three treatments i.e., 1.80 percent (control), 1.89 percent (blanched), and 2.23 percent (autoclaved). However, kinnow without albedo had 4.39, 4.45, and 4.50 percent in control, blanched, and autoclaved, respectively. Further, it was seen that among all peels there was an increase in SDF after treatment which might be due to the fact that heat treatment content of soluble fiber is increased as cell walls get loosened with heat which increases water soluble fiber content. Overall, there was a significant ($p < 0.05$) increase in the TDF after the blanching and autoclaving treatment (Table 3). As per previous findings, blanched items had greater dietary fiber content. This could be because low molecular weight nutrients including sugar, vitamins, and minerals leak out of plant cells into the blanched water significantly increased the amount of dietary fiber [26].

Prebiotic potential of fruit peels

By combining probiotic culture *Lactobacillus plantarum* with fruit peel powders at 0.1 percent concentration, probiotic growth media were created and stored in the refrigerator for 28 days. Probiotic viability was analyzed over the entire period of 28 days af-

ter 7 days under refrigerated storage at 4°C. There was an overall increase in log CFU/ml in blanched fruit peels powder-containing media followed by autoclaved fruit peels powder-containing media. Thus, control fruit peel powders containing media had the lowest log CFU/ml. The highest CFU/ml was observed in both blanched and autoclaved kinnow without albedo, which was 7.61 CFU/ml on 0 days but significantly decreased ($p < 0.05$) for 28 days (Table 4). The corresponding figures were observed as 6.69 and 7.20 CFU/ml at end of storage periods for blanched and autoclaved, respectively. Food Safety and Standards Authority of India (FSSAI) has mentioned that a minimum of 10⁸ CFU/ml active, viable, and live microorganisms must be present in the probiotic food at the time of consumption [27].

In recent studies, the growth kinetics of *Lactobacillus acidophilus* and *Lactobacillus plantarum* was observed with the absence or presence of watermelon and banana peel powder in milk. The study revealed that there was a decrease in log value within storage period of 42 days, but the survival of lactobacillus acidophilus was more supported by watermelon peel powder instead of banana peel powder [28]. The difference in the probiotic count at the end of four weeks of refrigerated storage was found statistically significant ($p < 0.05$). Lower the pH, lower the content of sugar in

Fruit Peel	Treatments	0 th day	7 th day	14 th day	21 st day	28 th day
Kinnow with albedo	Control	7.08 ± 0.01 ^{aZ}	7.08 ± 0.01 ^{aZ}	7.02 ± 0.01 ^{bZ}	6.94 ± 0.01 ^{cX}	6.79 ± 0.02 ^{dX}
	Blanched	7.26 ± 0.01 ^{aY}	7.19 ± 0.02 ^{bY}	7.06 ± 0.02 ^{cY}	6.81 ± 0.01 ^{deY}	6.63 ± 0.02 ^{eZ}
	Autoclaved	7.53 ± 0.01 ^{aX}	7.51 ± 0.02 ^{bX}	7.18 ± 0.01 ^{cX}	6.92 ± 0.02 ^{dX}	6.70 ± 0.01 ^{eY}
Kinnow without albedo	Control	7.60 ± 0.01 ^{aX}	7.57 ± 0.01 ^{bX}	7.48 ± 0.02 ^{cX}	7.41 ± 0.01 ^{dX}	7.19 ± 0.01 ^{eX}
	Blanched	7.61 ± 0.02 ^{aX}	7.60 ± 0.02 ^{aX}	7.47 ± 0.03 ^{bX}	7.29 ± 0.01 ^{cY}	6.96 ± 0.02 ^{dY}
	Autoclaved	7.61 ± 0.01 ^{aX}	7.60 ± 0.02 ^{aX}	7.51 ± 0.01 ^{bX}	7.40 ± 0.002 ^{cX}	7.20 ± 0.02 ^{dX}
Orange with albedo	Control	7.37 ± 0.02 ^{aX}	7.32 ± 0.01 ^{abX}	7.26 ± 0.05 ^{bX}	7.06 ± 0.02 ^{cX}	6.99 ± 0.04 ^{cX}
	Blanched	7.23 ± 0.01 ^{aY}	7.18 ± 0.01 ^{bZ}	7.07 ± 0.01 ^{cY}	6.72 ± 0.03 ^{dY}	6.40 ± 0.06 ^{eY}
	Autoclaved	7.25 ± 0.02 ^{aY}	7.21 ± 0.01 ^{abY}	7.07 ± 0.02 ^{cY}	6.67 ± 0.02 ^{dY}	6.16 ± 0.12 ^{eZ}
Orange without albedo	Control	7.08 ± 0.01 ^{aX}	7.04 ± 0.02 ^{abX}	6.98 ± 0.01 ^{bcX}	6.75 ± 0.02 ^{cX}	6.67 ± 0.03 ^{dX}
	Blanched	7.06 ± 0.02 ^{aX}	6.97 ± 0.01 ^{bY}	6.89 ± 0.01 ^{cZ}	6.69 ± 0.01 ^{dY}	6.58 ± 0.05 ^{deXY}
	Autoclaved	7.01 ± 0.01 ^{aY}	6.97 ± 0.01 ^{bY}	6.92 ± 0.01 ^{cY}	6.88 ± 0.01 ^{dZ}	6.65 ± 0.03 ^{eX}
Lemon	Control	6.91 ± 0.01 ^{aX}	6.79 ± 0.01 ^{Box}	6.73 ± 0.01 ^{cX}	6.51 ± 0.04 ^{dX}	6.33 ± 0.08 ^{eX}
	Blanched	6.79 ± 0.02 ^{aY}	6.74 ± 0.01 ^{abY}	6.62 ± 0.04 ^{cY}	6.46 ± 0.02 ^{dX}	5.73 ± 0.05 ^{eY}
	Autoclaved	6.77 ± 0.01 ^{aY}	6.71 ± 0.01 ^{bZ}	6.44 ± 0.04 ^{cZ}	5.95 ± 0.06 ^d	5.38 ± 0.12 ^{eZ}

Table 4: Effect of storage on the growth rate of modified media containing fruit peels (CFU/ml).

Values represent mean ± SD, (n = 3), Values having different superscripts from X, Y, Z are significantly (p < 0.05) different from each other among treatments, Values having different superscripts from a, b, c to e are significantly different from each other day wise in rows.

fruit peels and increase the titratable acidity which might decline the viability count of probiotic media. There may be few reasons which decrease the growth kinetics, it might be due to antibacterial compounds, hydrogen ion molecular oxygen, and increases in organic acids [29,30]. Studies also reported that there was a significant reduction (P < 0.05) during storage of inoculated smoothie with different formulations thus reduction in the probiotic viability of the beverages which counted between 6.93 log CFU/ml and 5.49 log CFU/ml⁻¹ [31].

Conclusion

The study concluded that the maximum total phenolic and flavonoid content was found in Orange without albedo (14.07 mg GAE/g) and (10.02 mg QE/g), respectively. Orange without albedo exhibited the highest antioxidant activity i.e., 82.97 percent RSA whereas orange with albedo showed maximum FRAP activity which was 17.32 mg FeSO₄. The antioxidant potential of all fruit peels decreased after autoclaving but there was no significant difference between values of total antioxidants and antioxidant activity. The highest amount of total dietary fiber content was found in orange with albedo peel powder with an average value of 51.99 percent. However, kinnow peel without albedo showed the highest value for probiotic viability i.e., 7.61 CFU/ml. The findings of the study recommended that fruit peels with highest prebiotic potential may be used to develop prebiotic rich products. Further, *in vivo* trials may be performed to assess probiotic adherence and viability of the developed products in human cell lines.

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Conflict of Interest

There was no conflict of interest.

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