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Banana (*Musa acuminata*), Orange (*Citrus reticulata*), and Watermelon (*Citrullus lanatus*) Peels as Prebiotic

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ABSTRACT

Fruit waste is being studied as a non-conventional alternative source of nutritional and mineral content that might be employed as functional food ingredients. This study aims to identify the 1) proximate and mineral composition of banana, orange and watermelon waste powder subjected to different drying methods; 2) prebiotic potential of the fruit waste powder (FWP) in growth enhancement of the probiotic *Lactobacillus casei*. The fruit peels were processed by two methods: freeze-dried and oven-dried. All FWP was sterilised and milled into particle size <180µm. The proximate (total ash, crude protein, crude fat, crude fibre), mineral (Ca, Zn, Na, K, Mg, Cu) profiling was analysed in triplicate according to standard. Prebiotic activities of FWP were determined through the growth of *L. casei* analysed. Significant differences (p<0.05) result was observed between the proximate and mineral parameters in all FWP. Watermelon FWP had the highest moisture, ash, sodium, potassium, phosphorus, and zinc content, while banana FWP contained the highest crude protein, crude fat, and magnesium content. Both banana and watermelon FWP were found to exhibit high crude fibre content. The orange WP was reported with the highest carbohydrate, calcium, and copper content. Although significant differences (p<0.05) in composition were noted, the oven and freeze-drying methods employed showed no pronounced effect. Calcium, copper content (all FWP), sodium and phosphorus (watermelon FWP), phosphorus (banana FWP) examined highly exceeded the recommended dietary allowance (RDA) limit. Banana FWP showed the highest *L. casei* net growth of $\log_{10}$ 8.28±0.02– 8.36±0.01 CFU/mL and 91.61–98.66% of survival rate, thus showing its potential as prebiotic agents among other FWP. All types of FWP showed significant difference (p<0.05) in bacterial growth except for oven-dried orange FWP. Overall, the results revealed that all these fruit wastes could be exploited for the nutrient and value-added potential in food formulations due to their inexpensiveness, natural, safe, and environmental friendliness.

Keywords: Fruit waste powder, *Lactobacillus*, mineral, prebiotics, proximate

INTRODUCTION

The increasing demand for fruit consumption in today's health-conscious society has brought a parallel increase in fruit waste produced. Statistics report that approximately 100 kg per week of fruit waste such as peels and seeds are thrown in residential or municipal waste, thus accounting as the second largest type of waste produced globally (Rohana *et al*., 2019). Fruit waste is disposed in a raw condition with high moisture content which will be biodegraded naturally. However, this process may cause an unpleasant odour. Long-term environmental disposition of these fruit wastes may lead to greenhouse gas emissions into the atmosphere as well as provide breeding ground for bacteria, pathogens, pests, and mice. Thus, turning these fruit wastes into functional ingredients for food is encouraged.

Agro-industrial by-products, especially fruit waste, are an essential source of bio-compounds that can be used as functional food ingredients. It has been shown to be a good source of fibre, prebiotics, and antioxidants (Zahid *et al*., 2021). The bioavailability of these nutrients is highly dependent on recovery procedures such as drying methods used in powder manufacturing. The drying procedures, specifically oven and freeze-drying, have a significant impact on the functional features of the dried powder, including solubility, flow characteristics, water- and oil-holding capacity, and foaming capacity (Mirhosseini & Amid, 2013). Additionally, Sogi *et al.* (2013) suggested that freeze-drying provides benefits over traditional drying processes in terms of solubility, nutrient preservation, etc. Studies were done in promoting the development of probiotic lifeforms such as improving the fiber-rich fractions of herbs in India (Kaur *et al*., 2021), cereal in Australia (Salgaço *et al*., 2021), banana, passion fruit, apple in Brazil (do Espírito Santo *et al*., 2012), and pomegranate peels obtained from a local market in Egypt (Al-Hindi & Abd El Ghani, 2020). The interest in using fruit wastes in the manufacturing industry has begun producing high nutritional content supplements, enzyme and energy production agents commercially. Previous research has proved that fruit peels, skin, and pulp contain nutritional components like carbohydrates, proteins, fibres (Garcia-Amezquita *et al.*, 2018) and mineral components such as potash, calcium, iron, and zinc (Morais *et al*., 2017). Therefore, fruit waste is a potential source of natural prebiotics obtained from unconventional materials to stimulate probiotic development. This study focused on three types of widely consumed fruits, namely banana (*Musa acuminata*), orange (*Citrus reticulata*), and watermelon (*Citrullus lanatus*). Most of the citrus fruit pulp and peel are proven to be valuable sources of macro- and micronutrients (Czech *et al*., 2020). Banana peels were reported as a good source of crude fibre, carbohydrates, crude protein, and ash which can be utilised as a base material for animal feed (Pyar & Peh, 2018). They contain rich sources of dietary fibre and are particularly in high demand in most food processing industries due to their low market price and availability in huge quantities.

Different drying methods applied on the fruit waste may affect the nutritional and mineral contents. In this study, the fruit waste is processed using two drying methods (oven and freeze-drying). Both oven and freeze-drying are a dehydration technique based on the suppression of the water content reside in the wastes. Freeze-drying begins with freezing fruit waste in order to be subjected to vacuum pressure with the consequent sublimation and desorption of the water. Freeze-drying operates at low temperatures, thus preserving the taste, colour, and appearance characteristics. Moreover, freeze drying is able to minimise the degradation of thermolabile compounds which represent the nutritional value of the fruits (Barbosa-Canovas *et al*., 2005). Freeze drying process may take longer time to complete. Unlike oven drying which operates at slightly high temperature (60 °C) and shorter process duration. However, high temperature may affect the nutritional and mineral content in the fruit waste.

In order to show good functional food properties, important industrial probiotics such as *Lactobacillus casei* is chosen for testing in the study. It has been widely used as starter cultures for dairy food products manufactured, traditional additives, and as probiotics for human health benefits in therapeutic application (Karami *et al.*, 2017). *Lactobacillus casei* strains consist of *L. casei*, *L. paracasei*, and *L. rhamnosus*, where they are applied to ferment dairy food products for enhancement of taste and texture (Bis-Souza *et al*., 2020). Researchers have discovered numerous bioactive metabolites produced by *L. casei* to treat or prevent diseases such as colon cancer and obesity (Tripathy *et al*., 2021). Therefore, we hypothesise that *L. casei* will respond positively to the FWP as a potential prebiotic substrate due to its nutrient bioavailability.

MATERIALS AND METHODS

Chemicals and Standards

The reagents were: sulfuric acid (Emsure®, Malaysia), sodium hydroxide (Qrec, Malaysia), 2% of boric acid (Merck, Germany), 0.01 M hydrochloric acid (HmbG® Chemicals, Malaysia), hydrochloric acid (Merck, Germany), and nitric acid (Emsure®, Germany). Standard solutions of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), copper (Cu), zinc (Zn), and phosphorus (P): 100 ppm (stock solution) obtained from 1000 µg/mL (mineral element) in 2% (v/v) HNO_3 (diluted from concentrated nitric acid, PerkinElmer Pure, USA), potassium dihydrogen phosphate (Merck, Germany), ammonium molybdate (Systerm, ChemAR, Malaysia), concentrated sulfuric acid (Fisher Scientific, Malaysia), potassium antimony (Merck, Malaysia), and ascorbic acid (Merck, China) were used. All solutions were analytical reagent grade and prepared accordingly using Milli-Q water.

Fruit Source

Whole banana (*Musa acuminata*) of variety Cavendish, orange (*Citrus reticulata*) variety Mandarin, and watermelon (*Citrullus lanatus*) variety of Carolina Cross were purchased from a local supermarket (Farley Supermarket Bintulu, Sarawak, Malaysia).

Preparation of FWP

Fresh fruits were washed and allowed to dry at room temperature. The peels were removed and prepared by boiling the peel cuts in hot water (± 90 °C) for 30 min to inactivate potential pathogens and enzymatic reactions. The peels were dried using two different methods, i.e., Group 1: dried in an oven (Memmert UNB500, Germany) at 60 °C until a constant weight was achieved, and Group 2: dried in a freeze dryer (Ilshin freeze dryer system TFD5503, Korea). The peels were then milled to fine powders using grinders and sieved (Cooper Research Technology, particle size <180 µm), then sterilised under UV irradiation for 30 min. The FWP were stored in airtight containers at ambient temperature (25 to 30 °C) until further analysis as described by do Espírito Santo *et al*. (2012) (Figure 1).

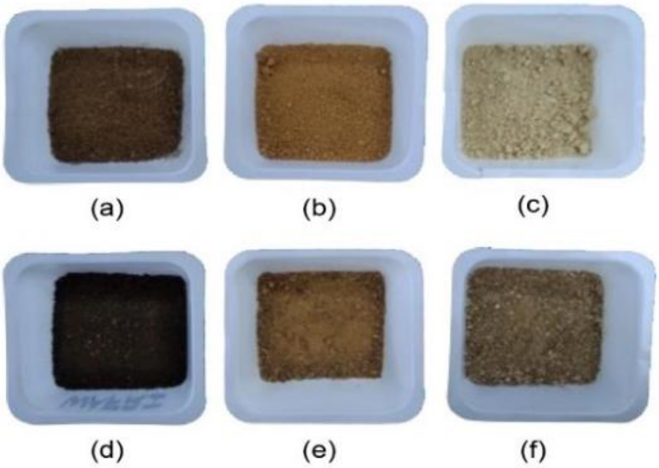

Figure 1. The FWPs used in this study (after milling process). **(**a) Freeze-dried banana peel; (b) freeze-dried orange peel; (c) freeze-dried watermelon peel; (d) oven-dried banana peel; (e) oven-dried orange peel; (f) oven-dried watermelon peel

Proximate Composition Analysis

Moisture content was determined after oven-drying (Memmert UNB500, Germany) to a constant weight at 100 °C. Total ash, crude protein, crude fat, and crude fibre were analysed according to the Association of Official Analytical Chemists methods (AOAC, 2005). The carbohydrates content was determined by the difference as follows: Carbohydrate % = 100 – (moisture % + crude protein % + ash % + crude fat % + crude fibre %). All analyses were carried out in triplicate, and values were expressed as percentages on a dry basis (% DW), except for moisture, which was expressed as a percentage on a wet basis (% WB).

Mineral Composition Analysis

The digestion of the samples was carried out by drying a 1 g of FWP and crucible in a muffle furnace (Barnstead 62700, USA) at 520 °C for 4 to 5 hr until whitish or greyish ash was obtained. The ash was treated with 2 mL of concentrated hydrochloric acid (Merck, Germany) and 20% nitric acid (Emsure®, Germany) until complete dissolution of the ash, which was then filtered. After the sample had reached room temperature, the solution was transferred into a volumetric flask and made up to 100 ml using sterile distilled water before submission to atomic absorption spectrophotometry (AAS) (AOAC, 2005).

The determination of Ca, Zn, Na, K, Mg, and Cu contents was performed using an AAS

(AAnalyst 800, Perkin Elmer Instrument, Norwalk, CT). Calibration curves for each element were plotted using standard mineral diluted with sterile distilled water. Meanwhile, P analysis was calculated by measuring the blue indicator formed when the ash solution was treated with ammonium molybdate using UV-Vis Spectrophotometer (Perkin Elmer Lambda 25, USA) at 882 nm. All analyses were performed in triplicate; the results were expressed in milligrams per 100 g of sample on a dry basis (mg/100 g DW).

Culture Acquisition

Starter strain of *Lactobacillus casei* (*L. casei*) ATCC 393 was obtained from Chr. Hansen A/S as frozen direct-to-vat starters (DVS) (Gayrettepe, Kuala Lumpur, Malaysia). *Lactobacillus casei* was cultivated in 46 ml of Man Rogosa Sharpe, (MRS) broth (Oxoid, UK) according to the method as described by Lingoh *et al*. (2020). The strain was preserved in 40% (v/v) glycerol stock at -80 °C for further analysis.

Total Viability of *Lactobacillus casei* on Modified MRS Media

The prebiotic activity of the fruits waste was determined through the growth of *L. casei* as described with minor modification (Lingoh *et al*., 2020). The modified MRS broth which is referred to as mMRS was prepared in adjusted formulations of MRS broth (Oxoid, UK) by mixing of 20 g/L of FWP sample, 10 g/L bacteriological peptone (Merck, Germany), 8 g/L Lab Lemco powder (Himedia, India), 4/L yeast extract (Oxoid, UK), 1 g/L of Tween 80 (BDH Chemicals, Poole, England), 2 g/L di-potassium hydrogen phosphate (Merck, Germany), 5 g/L sodium acetate trihydrate (Nacalai Tesque, Japan), 2 g/L of ammonium citrate tribasic (Himedia, India), 0.2 g/L of magnesium sulphate heptahydrate (Fisher Bioreagents, India), and 0.05 g/L of manganese sulphate tetrahydrate (Merck, Germany) in Milli-Q water. The steps were repeated by using glucose (Sigma-Aldrich, UK) as a positive control and non-glucose added as a negative control. The pH of the mMRS broth medium was adjusted to 6.2±0.2 at 25 °C before sterilisation at 121 °C for 15 min. The 1% (v/v) overnight grown probiotic culture *L. casei* (1 – 2 x 10^9 cfu/mL) was aseptically inoculated into the sterile mMRS broth and incubated at 37 °C for 24 hr with agitation (120 rpm). The bacterial colonies were plated on MRS agar in anaerobic conditions and incubated at 37 °C for 48 hr. All samples including positive and negative control were done in duplicate. The preparation of mMRS broth used in this study was tabulated (Table 1). The bacteria viable cell count was determined using the following formula as Eq. 1,

$$\text{CFU/mL} = \frac{\text{number of colonies x dilution factor}}{\text{volume culture plate}} \quad \text{Eq.1}$$

Table 1. Preparation of modified De Man, Rogosa and Sharpe (mMRS) broth

Sample and treatments	Growth medium component
Banana (FD) Banana (OD) Orange (FD) Orange (OD) Watermelon (FD) Watermelon (OD) Glucose (Positive control)	mMRS broth, *L. casei* starter strain, and treatment substrate respectively.
Negative control	mMRS broth and *L. casei* starter strain.

Note: FD represents freeze-dried and OD represents oven-dried.

Survivability of Probiotic Growth

The survival counts of the probiotic cell was enumerated using modified method as described by Lingoh *et al*. (2020). The test was performed in duplicate including positive (medium with glucose) and negative (medium without glucose) controls. The colony forming unit (CFU) was recorded at 0 hr and 3 hr of anaerobic incubation at 37 °C. The Eq. 2 was applied to measure the survival rates of *L. casei* in adjusted conditions:

$$\text{Survival rate (\%)} = \frac{\text{Final – Initial (log CFU/mL)}}{\text{Initial (CFU/mL)}} \text{ x 100} \quad \text{Eq. 2}$$

Statistical Analysis

Results were reported as means ± standard deviation (SD). Data were analysed by one-way analysis of variance (ANOVA). The Tukey's test, at $p<0.05$, was used to assess significant differences between means of samples.

RESULTS

Proximate Composition of FWP

Table 2 shows the proximate composition of moisture, total ash, crude protein, crude fibre, crude fat, and carbohydrate contents of freeze and oven dried FWP of banana, orange, and watermelon. Significant differences (p<0.05) were observed between the proximate parameters in all FWP. The watermelon FWP was recorded with the highest content of moisture (7.96±0.22 to 11.06±0.92%/100 g WB) and ash (17.99±0.25 to 18.63±0.27%/100 g DW). The banana WP was observed with the highest content of crude protein (1.53±0.18 to 1.54±0.30%/100 g DW) and crude fat (4.73±0.23 to 5.26±0.23%/100 g DW). Both banana and watermelon FWP was presented with high crude fibre content (14.65±0.37 to 21.15±4.80%/100 g DW; 21.01±0.68 to 22.00±0.35%/100 g DW). The highest carbohydrate content was noticed in orange WP (80.26±1.28 to 83.04±0.40%/100 g DW). Although significant differences (p<0.05) in composition noted, however, freeze and oven-drying methods employed were showing no pronounced effect. Trend analysis of the study reported carbohydrate as the highest nutritional content while crude protein as the lowest nutritional content detected in all the FWP except for watermelon FWP. The watermelon FWP has crude fat as the lowest nutritional content.

Mineral Analysis of FWP

Table 3 shows the mineral composition of calcium (Ca), zinc (Zn), sodium (Na), potassium (K), magnesium (Mg), copper (Cu), and phosphorus (P) of freeze and oven dried FWP of banana, orange, and watermelon. Significant differences (p<0.05) were observed between the mineral contents of the three different FWP. The banana FWP was revealed with the highest magnesium content (29.34±1.81 to 45.3±3.03 mg/100 g). The highest calcium and copper content was found in orange FWP (2341.56±220.99 to 3239.17±358.34 mg/100 g; 17.08±2.03 to 32.26±1.41 mg/100 g). The watermelon FWP was reported with the highest zinc, sodium, potassium, phosphorus content (0.32±0.04 to 0.33±0.01 mg/100 g; 2301.04±426.37 to 2727.54±247.10 mg/100 g; 279.77±12.04 to 330.28±34.75 mg/100 g; 27.26±1.56 to 29.35±5.57 mg/100 g), respectively. Although significant differences (p<0.05) in minerals were noted, however, oven and freeze-drying methods employed were showing no pronounced effect. Both drying methods did not show significant different in the FWP nutritional content. However, freeze-drying banana and watermelon FWP lower calcium, sodium, and phosphorus content. Besides, copper and magnesium content had displayed an opposite trend between banana FWP and orange FWP. Trend analysis of banana and orange FWP showed the highest mineral content as calcium; sodium for watermelon FWP; and the lowest as zinc except for freeze dried banana FWP.

Calcium and copper content examined for all the FWP in this study have highly exceeded the RDA limit (1000-12000 mg/day; 0.90 mg/day) (RDA, 1989). While, sodium and phosphorus content for watermelon FWP have highly exceeded the RDA limit (1500 mg/day, 700 mg/day) (RDA, 1989). Lastly, phosphorus content reported for banana FWP has slightly exceeded the RDA limit (700 mg/day) (RDA, 1989). However, the results highlighted that the quantity of mineral composition in the same FW from freeze- and oven-dried approaches is in a comparable range.

Total Viability of *Lactobacillus casei* Enumeration

Figure 2 shows the viable counts (log_{10} CFU/mL) of *L. casei* on the mMRS broth enriched with FWP (banana, orange, and watermelon peels) as the main substrate. The mMRS broth with glucose as positive control and without glucose as negative control. Result revealed that oven and freeze-dried banana FWP indicated a higher population of bacterial growth at log_{10} 8.36±0.01 CFU/mL and log_{10} 8.28±0.02 CFU/mL, respectively. Meanwhile, oven-dried orange FWP showed the lowest population of bacterial growth with log_{10} 7.68±0.01 CFU/mL. All the FWP showed more efficient bacterial growth performance than the positive control (log_{10} 7.76±0.3 CFU/mL) except for oven-dried orange FWP. Bacterial colony count of freeze- and oven-dried banana FWP were significantly different ($p<0.05$) from the control. However, there is no significant difference observed in the bacterial growth between watermelon and banana FWP.

Survivability of Probiotic Growth

Figure 3 reveals the survival rate percentage (%) of *L. casei* cultivated from MRS agar after 3 hours of incubation period by using mMRS broth. Overall, the results showed that only banana FWP displayed a high survival rate of more than 80%. Freeze-dried banana FWP showed 98.66% and oven-dried banana FWP showed 91.61% of survival rate, thus showing their potential to be utilised as potential prebiotic agents among other FWP. Banana FWP showed significant difference ($p<0.05$) in the survival rates of *L. casei* between orange FWP, watermelon FWP, positive, and negative control. Meanwhile, a lower percentage of survival rates was expressed by the oven-dried orange FWP at 22.26% is similar to the positive control value of 21.25%. There are no significant survival rates among orange FWP, and positive control. On the other hand, the survival rates in negative control displayed significantly different with all FWP and positive control. Hence, considered as non-survival throughout the 3 hours incubation period due to no bacterial growth.

DISCUSSION

The findings indicated that watermelon FWP is rich in moisture content compared to other FWP evaluated in this study. Water content is essentially the regulating mechanism in the life span of the living commodity. Food by-products with lower water content can be stored for longer periods and, therefore, capable of retaining their quality. Between the two processing methods, freeze-drying is suitable for preserving watermelon and orange FWP, but not for banana FWP (Table 2). The values determined in this study were slightly lower than Ho *et al*. (2016) and Morais *et al*. (2021), who reported 14.98±0.21% and 13.9±0.2% FWB in freeze-dried and oven-dried watermelon peel. However, the moisture content of banana and orange FWP obtained was higher than previously reported studies, as 9.80±0.0% and 3.16±0.123% WB, respectively (Pathak *et al*., 2017). The differences in moisture value may be attributed to variations in fruit varieties or geographical factors. Besides, the drying method applied in the study may affect the moisture content of the FWP. Freeze-drying methods may cause freeze injury to the plant cells which could mechanically damage them during the freezing process, leading to an increase in the amount of moisture in FWP (Akubor & John, 2012). Oven-drying removes the moisture of FWP through heat, which has proved to improve food digestibility, nutrients concentration, and nutrient availability in FWP (Morris *et al*., 2004). In order to maintain the good condition of FWP, the standard moisture content is set as less than 14% to minimise microbial growth and improve shelf life (Mokhtar *et al*., 2018). In the study, either freeze or oven-drying methods were able to achieve the standard moisture level.

Elimination of water and organic matter by heating of food will produce inorganic residue, ash. Ash content of a sample is the indication of the total amount of minerals present in the test sample (Omotoso & Adedire, 2007). Based on the results, watermelon FWP showed the highest ash content (Table 2), indicating the presence of a substantial amount of minerals in the fruit peels. Liu (2019) has highlighted that a significantly increased ash composition was correlated with the increase of mineral contents in certain foods. Morris *et al.* (2004) explained that high ash content was mostly related to moisture reduction, which helps to enhance nutrient concentration. However, these opposed the result obtained in this study.

Banana FWP recorded the highest crude protein content among all evaluated FWP. The crude protein levels of all FWP determined in this study were lower compared with the study done by Hassan *et al*. (2018). They reported 1.95±0.14%/100 g DW of crude protein in banana peels is higher compared to 1.53±0.18%/100 g DW acquired in the present study. Trend analysis reported watermelon and orange FWP were low in crude protein. The value obtained from the present study was lower compared to the previous study done by M'hiri *et al*. (2016) for citrus peels, with a range of 1.79 to 9.06% DW. According to Mæhre *et al.* (2018), the reduction in protein content could be attributed to the rising temperatures during the crude protein analysis of the Kjeldahl method. However, it is important to highlight that the crude protein value in this study contained lower

Table 2. Proximate composition (% per 100 g dry weight): moisture, total ash, crude protein, crude fibre, crude fat, and carbohydrate contents of freeze and oven drying fruit wastes powder (FWP) of banana, orange, and watermelon

Parameters	Sample (MEAN ± STD)						F value	*p*-value
	Banana		Orange		Watermelon			
	FD	OD	FD	OD	FD	OD		
Moisture§	1.99 ± 0.02 c	1.81 ± 0.07 cd	0.93 ± 0.06 d	1.82 ± 0.07 cd	7.96 ± 0.22 b	11.06 ± 0.92 a	271.88	<.0001
Ash	14.33 ± 0.23 b	16.62 ± 2.04 ab	3.62 ± 0.16 c	3.82 ± 0.70 c	17.99 ± 0.25 a	18.63 ± 0.27 a	133.75	<.0001
Crude protein	1.54 ± 0.30 a	1.53 ± 0.18 a	0.51 ± 0.08 c	0.53 ± 0.15 c	0.82 ± 0.33 bc	1.40 ± 0.08 ab	10.87	0.0006
Crude fibre	21.15 ± 4.80 a	14.65 ± 0.37 b	10.78 ± 0.18 b	12.62 ± 0.39 b	22.00 ± 0.35 a	21.01 ± 0.68 a	12.51	0.0003
Crude fat	5.26 ± 0.23 a	4.73 ± 0.23 a	1.20 ± 0.20 b	1.03 ± 0.31 b	0.20 ± 0.07 b	0.83 ± 0.01 b	54.12	<.0001
Carbohydrate	56.4 ± 4.88 bc	61.32 ± 2.20 b	83.04 ± 0.40 a	80.26 ± 1.28 a	51.13 ± 0.31 cd	47.74 ± 1.55 d	77.85	<.0001
Trend analysis	C > Fi > A > F > P	C > A > Fi > F > P	C > Fi > A > F > P	C > Fi > A > F > P	C > Fi > A > P > F	C > Fi > A > P > F		

Note: § Wet weight basis. a, b, c, d Different superscript letters in the same row reveal statistical difference at $p < 0.05$ (n = 3). FD represents freeze-dried, and OD represents oven-dried.

Table 3. Mineral composition (mg/100 g dry weight) of calcium (Ca), zinc (Zn), sodium (Na), potassium (K), magnesium (Mg), copper (Cu), and phosphorus (P) of freeze and oven drying fruit wastes powder (FWP) of banana, orange, and watermelon

Parameters	Sample (MEAN ±STD)						F value	*p*-value	RDA average index value
	Banana		Orange		Watermelon				
	FD	OD	FD	OD	FD	OD			
Calcium	2147.2±122.8 b	2135.89±142.89 b	2341.56±220.99 b	3239.17±358.34 a	2241.06±123.56 b	2183.23±46.55 b	8.81	0.0014	1000-1200
Magnesium	45.3±3.03 a	29.34±1.81 cd	17.56±1.42 e	26.51±3.00 d	34.57±3.33 bc	35.99±3.30 b	90.70	<.0001	310-420
Sodium	1050.41±354.34 b	1183.10±269.32 b	842.32±203.97 b	826.42±200.84 b	2301.04±426.37 a	2727.54±247.10 a	14.97	0.0001	1500
Potassium	208.23±5.73 c	240.73±24.63 bc	33.14±7.71 d	29.42±4.00 d	279.77±12.04 ab	330.28±34.75 a	92.85	<.0001	2600-3400
Phosphorus	569.66±24.71 c	823.20±69.87 b	403.0±48.64 c	343.28±105.65 c	1066.09±145.39 b	1405.81±74.45 a	47.37	<.0001	700
Zinc	0.12±0.02 b	0.12±0.01 b	0.10±0.01 b	0.11±0.03 b	0.32±0.04 a	0.33±0.01 a	45.81	<.0001	8-10
Copper	26.7±2.51 ab	21.63±1.62 bc	17.08±2.03 c	32.26±1.41 a	27.26±1.56 ab	29.35±1.57 ab	8.21	0.0018	0.9
Trend analysis	Ca > Na > P > K > Mg > Zn > Cu	Ca > Na > P > K > Mg > Cu > Zn	Ca > Na > P > K > Cu > Mg > Zn	Ca > Na > K > P > Cu > Mg > Zn	Na > Ca > P > K > Mg > Cu > Zn	Na > Ca > P > K > Mg > Cu > Zn			

Note: a, b, c, d, e Different superscript letters in the same row reveal statistical significance at $p < 0.05$ (n = 3). FD represents freeze-dried, OD represents oven-dried, and RDA represent Recommended Dietary Allowances for adults established by USA National Institute of Health (NIH) for men and women, from 19 to 70 years old (mg/ day).

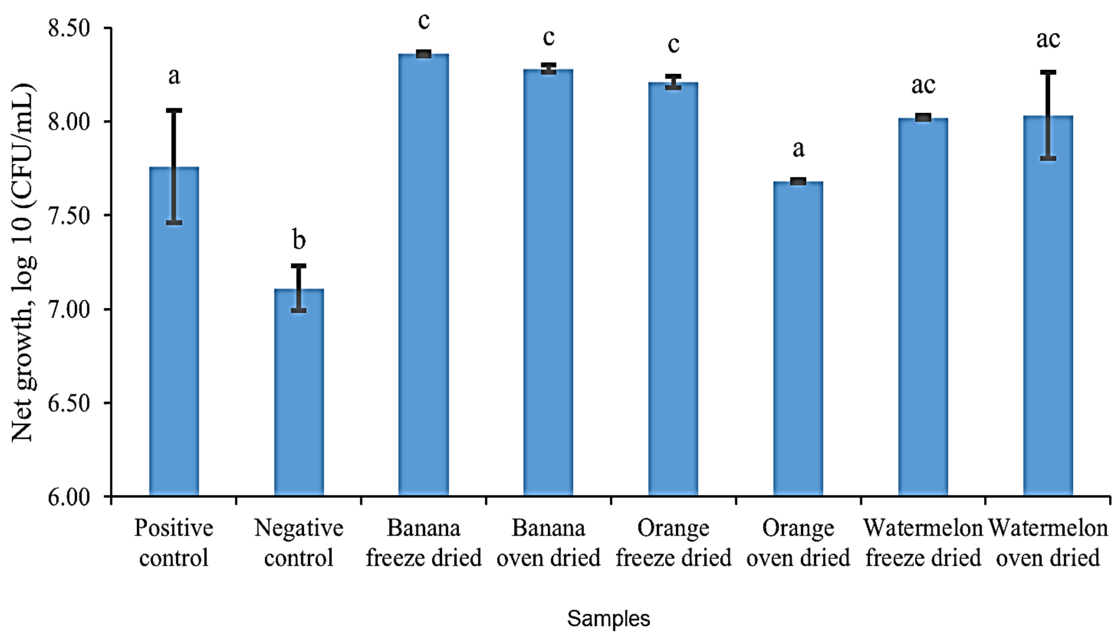

Figure 2. Viable counts ($\log_{10}$ CFU/mL) of *L. casei* on the mMRS broth enriched with FWP (banana, orange, and watermelon peels) as the main substrate. Data was presented in mean value (n = 3); a,b,c letters represents mean data with significant higher/lower growth count at the confidence level of 95%

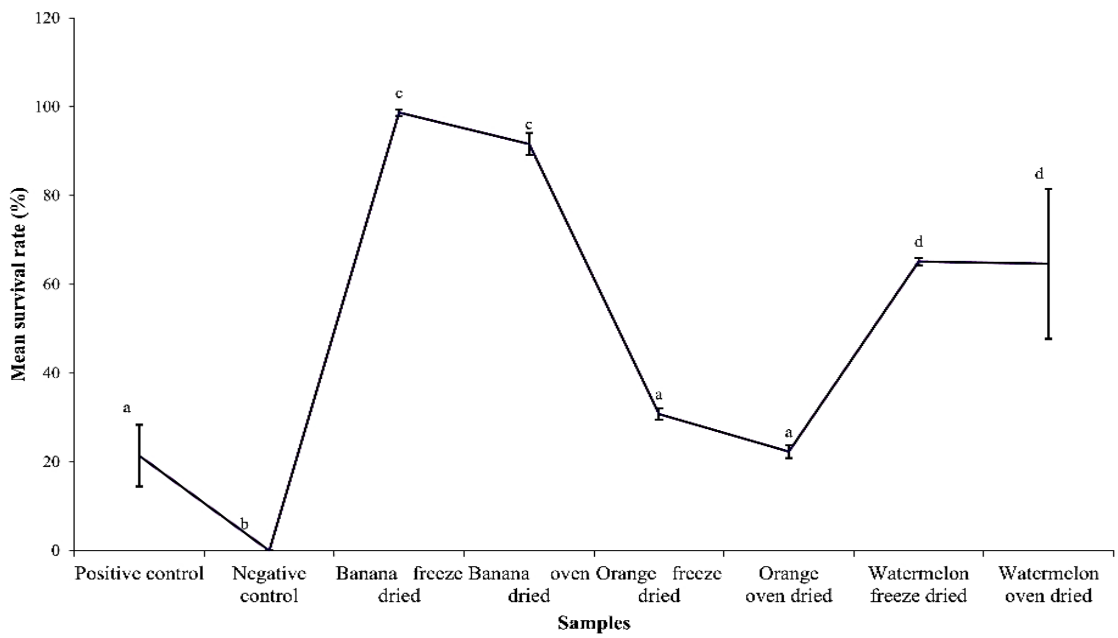

Figure 3. Survival rate (%) of *Lactobacillus casei* in MRS agar enriched with fruit waste powder after 3 hours of incubation period. Data was presented in mean value (n = 3); a,b,c letters represents mean data with significant higher/lower growth count at the confidence of 95%

nutrients compared to other proximate nutrient components. The result is expected since fruits are not particularly known as a major source of protein.

Crude fibre is an unhydrolysed compound composed of cellulose, lignin, and pentosane (Ho *et al*., 2016). Watermelon FWP had significantly higher crude fibre value of 21.01±0.68%/100 g compared to banana and orange FWP, 14.65±0.37%/100 g and 12.62±0.39%/100 g, respectively when applying the oven dry method. Romelle *et al.* (2016) reported 26.31±0.01%/100 g of crude fibre content in watermelon is slightly higher than the present study. Being a good source of food fibre, Akubor and John (2012) have stated that watermelon peel powder is beneficial to human nutrition by alleviating constipation issues, diabetes, colon cancer, and risk of coronary heart disease. Thus, watermelon FWP is a good source of food fibre supplements. Besides, fibre derived from FWP materials is believed to be proficient in stimulating the development and activities of *Lactobacilli* and *Bifidobacteria* (Davis & Milner, 2009).

The crude fat content of banana FWP obtained from the present study was lower than Hassan *et al*. (2018), who reported 5.93±0.13%/100 g in the same variety of banana. Different varieties of banana peels may show different crude fat levels. Banana FWP contains higher crude fat content if compared to other FWP, thus it will be more susceptible to oxidation in the environment (Ho *et al*., 2016). The study suggests that drying methods applied may also cause the reduction of crude fat content.

Carbohydrate is indispensable to the body as a source of energy. Hence, it is essential to ensure an adequate amount of intake. Carbohydrate contents recorded by banana, orange, and watermelon FWP in this study were significantly high. Orange FWP recorded the highest carbohydrate content, whilst the lowest was watermelon FWP. In another study conducted (Romelle *et al*., 2016), freeze-dried orange peels (29.77%) of variety Navel from India had a lower carbohydrate content compared to the present study. The difference may be attributed to the variation in the cultivar studied. It is crucial to highlight that freeze and oven-drying techniques did not significantly reduce the amount of nutrients because a similar proximate range was found in the same type of FWP. All FWP in the study showed high content of carbohydrate, thus all these FWP showed great potential to be applied as substrates for microbial growth.

Minerals are essential for the proper functioning of the various physiological processes in the human body. Basically, ash composition in a sample represents the overall amount of mineral content (Liu, 2019), which in turn, serves as an indicator for the presence of specific inorganic compounds. Minerals are less resistant to heat and less volatile compared to other food ingredients. Results showed that the ash contents of all the FWP were in similar range values. They were relatively high, which further confirmed the high mineral content (Table 3). According to Radha *et al.* (2021), a higher ash content in plants implies that the plant contains a huge amount of minerals that are essential for human health maintenance.

Orange FWP was reported with the highest calcium (Ca) and copper (Cu) content. Generally, Ca is crucial in bone formation and is strongly involved in regulating nerve and muscle activities. Based on our findings, orange FWP reported the highest Ca content while the lowest was obtained in banana FWP. Regardless, Ca was the most abundant mineral compared to other mineral elements analysed. However, the Ca contents obtained in this study were considered high, highly exceeding the RDA value. The Ca content reported in orange FWP was higher if compared with Romelle *et al*. (2016), who studied *Citrus sinensis* (variety Navel, India) peels (162.03±7.54 mg/100 g). Other variables, in addition to variety, such as state of ripeness, soil composition, soil quality, and irrigation regime can cause variations in the mineral and trace contents, even within different sections of the same fruit. All FWP can be considered natural sources of Cu. It is currently being used to cure a variety of diseases, including degenerative neurological disorders such as Alzheimer’s and Parkinson’s disease (Radha *et al*., 2021). The Cu content detected exceeded the RDA value of 0.9 mg/day. Our result was in concurrence with Edet *et al*. (2016), who reported a similar higher Cu content of 2.60±0.02 mg/100 g in *Citrus paradis*.

Watermelon FWP was reported with the most mineral content (zinc, sodium, potassium, phosphorus) in this study. Zinc (Zn) is crucial for cell proliferation, immune response, and in general, plays a significant role in human development. The zinc content detected in watermelon WP was different as reported by Gladvin *et al*. (2017) who analysed the same variety of watermelon (1.29 mg/100 g). Overall, Zn composition in FWP evaluated in this study was among the lowest compared to other mineral compositions. Sodium (Na) content emerged as the second most abundant composition after Ca. Both watermelon and melon (*Cucumis melo*) dried peels are known as good natural sources of Ca and Na (Morais *et al*., 2017). The Na content in freeze-dried watermelon WP evaluated in this study was reported higher in content than Morais *et al*. (2017), as 29.7±2.3 mg/100 g. Potassium (K) acts in concert with sodium in the human body. Higher K content and lower Na intake may help to prevent high blood pressure, which can lead to stroke, and heart problems. It is a primary intracellular mineral that is involved in muscle movement and heart muscles. The U.S. Food and Drug (2000) stated that a diet with a high K intake is beneficial for strong bone formation. In this study, our result has reported 330.28±34.75 mg/100 g in oven-dried watermelon FWP, which is higher than Rafiq *et al*. (2019), at 157.32±3.16 mg/100 g. Oven-dried orange FWP, on the other hand, had the lowest with 29.42±4.00 mg/100 g. Regardless, all three different FWPs are characterized by low K content, which is below the RDA (2600 to 3400 mg/day). The body's fundamental metabolism, bone mineralization, and resistance to tooth impact require adequate intake of phosphorus, P. In animals, Hassan *et al*. (2018) demonstrated that Ca and P deficiencies in animals result in decreased bone mineralisation, impaired bone strength, and slow growth. Generally, phosphates have been used for the preparation and storage of fruit as well as preserving the colour of fresh vegetables and fruits designed for immediate consumption. Both oven-dried banana and watermelon WP contained P levels exceeding the limit of the RDA of 700 mg/day. The watermelon peel evaluated by Gladvin *et al*. (2017) had a lower P content of 135.24 mg/100 g, but it emerged as the most abundant mineral component.

Magnesium (Mg) is an integral part of a balanced diet for humans. It is a sub-factor in several enzyme systems and is active in neurochemical transmission and muscle excitability together with Ca. It enhances the proper functioning of the digestive system, the preservation of bone and tooth tissue, and protein synthesis. The present study records a lower value of Mg content compared to the recommended daily requirement of 400 to 420 mg/100 g for males and 310 to 320 mg/day for females between the ages of 19 to 70 years old. The Mg content reported in banana FWP was lower than previously reported by Hassan *et al*. (2018), which is 44.50±0.08 mg/100 g of the similar variety.

After proximate and mineral profiling of the FWP was analysed, the FWP was tested for its prebiotic ability. Prebiotics must be screened for their capacity to facilitate the propagation of healthy microbes/probiotics in the human gastrointestinal tract. They act as non-digestible substrates for beneficial microbes, thereby supporting the host by supplying nutrition and vital micronutrients. Fortunately, this is a strain-specific symbiotic relationship combining probiotics and prebiotics. The symbiotic dietary intention is to shield the bacteria during their passage across the gastrointestinal tract (Rawi *et al*., 2020). Hence, assessing the probiotic bacteria growth and survival rate on alternate carbon sources provided are the vital criteria in choosing the best prebiotic in this study.

The result stated that all FWP in both drying methods significantly ($p<0.05$) increased the growth of *L. casei* in modified MRS broth. The banana is high in fibre, mainly cellulose substances, thus it enhances the viability of *L. casei* growth in a modified condition as a symbiotic relationship. A previous study done by do Espírito Santo *et al.* (2012) reported that banana peels (*Musa acuminata*) which are rich in fibre were able to promote the *L. delbrueckii* subsp. bulgaricus and *L. acidophilus* growth in yogurts with net growth $\log_{10}$ 9 CFU/mL after 24 hours of the incubation period. Watermelon FWP has been reported to have a mixture of different sugar content (non-reducing and reducing sugar), such as glucose, sucrose, and fructose which are able to promote five times faster growth of *Komagataeibacter hansenii* (Kumbhar *et al*., 2015). This indicated that glucose not only serves as a source of energy in the culture medium but also forms the precursor to produce cellulose and is being quickly utilised by the bacteria in early incubation phases. Kumbhar *et al*. (2015) stated that the bacterial cellulose grown in media substituted with watermelon and orange peel media had a higher consistency compared to bacterial cellulose produced in established culture media. Additionally, different sugars can cause various responses in microorganisms. This is essential for the formulation of symbiotic food, although there are other fermentable sugars and microorganisms. Chaouch and Benvenuti (2020) suggested that fruit by-products such as citrus fruits and watermelon peels have high content of pectin and can be used as an efficient prebiotic. These pectin-derived oligosaccharides have been reported with prebiotic characteristics in several studies (Wilkowska *et al.*, 2019). In this study, freeze-drying orange FWP showed slightly higher bacterial growth when compared with the watermelon FWP. According to Chatterjee *et al*. (2016), who reported that the lemon (*Citrus limetta*) peels showed significant growth of *L. casei* after 48 hr of incubation and contain the highest amount of pectin at 14.49% DW. This is equivalent to pectin content in orange FWP with varieties like those in the study conducted. Meanwhile, the result was in concurrence with Chatterjee *et al.* (2016), who reported that watermelon (*Citrullus lanatus*) peels contributed to the lowest *L. casei* growth due to the minimum amount of pectin concentration at 3.41% DW. It was found that apple pomace and citrus peel are the primary ingredients used to make commercial pectin to facilitate probiotic development and survival in the intestinal system under highly acidic to alkaline environments.

Oven-dried orange FWP was found to have a lower growth compared to other FWP. This is possibly caused by the drying method used for drying fruit by-products at temperatures exceeding 60 to 65 °C, which can result in changing structures with a rough or thick feel due to the plasticization effect of the low molecular weight sugars. Besides, it is not advised to use an oven-drying method as this might alter the dietary fibre structure (Morais *et al*., 2017). Adetoro *et al*. (2020) also reported that freeze-dried foods retained more soluble sugars than their oven-dried counterparts. Therefore, the fruit peels' pectin content and drying method are the key to the population growth of probiotic bacteria. Generally, bacteria can retrieve their preferred food and utilise it as a priority. Usually, bacteria choose carbohydrates in the form of simple sugar such as glucose as one of their preferred foods. The ability of *L. casei* to digest a carbohydrate was shown in negative control, with the lowest bacteria population growth as this may be attributed to the absence of glucose in the growth medium. Besides, Markowiak and Śliżewska (2017) stated that one of the most important growth factors of probiotic bacteria is their capacity to use glucose as a supply of energy. Thus, the functionalities of bacteria cannot be carried out without a proper digestion process of carbohydrates (glucose).

The behaviour and survival of the *Lactobacillus* strain were found to rely on the sources of carbon from which it was produced. Thus, among all the tested FWP, banana FWP promotes the best survival of *L. casei* with more than 80% survival rate, whilst lower was presented in orange FWP (Figure 2). The result was concurrent with previous research which stated that banana peels (*Musa acuminata*) promote the growth of *L. casei* and *L. rhamnosus* after 2 hours of incubation under *in vitro* gastrointestinal digestion conditions (Zahid *et al*., 2021). Thus, it is presented as one of the best among fruit peels for improving the viability of probiotic cells. More studies have been documented enhancing the survival of *L. acidophilus* and *L. casei* in MRS broth by applying the fermented milk formulated with citrus peels; pomegranate peels which showed 80% of survival rate after 4 hours of the incubation period in Egypt; and fruit peels extract in dairy products (e.g. yogurt) (Sendra *et al*., 2008). Nonetheless, studies further reported that probiotic populations declined in MRS broth during a prolonged storage method, where this may have affected the survival rate of oven-dried orange peel powder in this study. Despite this, *L. casei* has a high carbohydrate utilisation rate, thus allowing for survival and adaptation in a variety of environments. The result showed the increased growth of the tested probiotic strain was possibly due to prebiotic constituents and other growth factors in the fiber-rich fraction of FWP. It has been clearly shown that prebiotic carbohydrates of FWP tested were necessary for beneficial probiotic bacteria to improve survivability and growth development during adaptation to gastric conditions (Zahid *et al*., 2021). Banana FWP fits well into prebiotic criteria as a substrate to promote probiotics.

CONCLUSION

The study concluded that potential prebiotic properties are present in banana, orange, and watermelon peel powder, which are responsible for the enhancement of *L. casei* growth development. Significant differences in results were observed between the proximate and mineral parameters in all FWP. Banana FWP was found to fit well into prebiotic criteria as a substrate to promote probiotics net growth of $\log_{10}$ 8.36±0.01 - 8.28±0.02 CFU/mL and 91.61 - 98.66% of survival rate. All possible alternative carbohydrates chosen for this analysis were expected to have prebiotic properties and therefore will promote the development and metabolism of beneficial gut bacteria.

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