Available online: http://journal.ipb.ac.id/index.php/jgizipangan **J. Gizi Pangan, August 2024, 19(Supp.2):342-351**
Accredited based on DGHE, Republic of Indonesia 79/E/KPT/2023
ISSN 1978-1059 EISSN 2407-0920

Prebiotic Activity of Plants from *Cucurbitaceae* Family and In Vitro Fermentation by Gut Microbiota

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ABSTRACT

This study aims to examine the effect of Freeze-Dried Pumpkin Powder (FDPP), Freeze-Dried Winter Melon Powder (FDWMP), Freeze-Dried Rock Melon Powder (FDRMP), inulin, and D glucose on prebiotic activity score by in vitro fermentation. We also elucidate the changes in bacterial populations through batch culture fermentation using fecal samples from 5 healthy volunteers and In vitro fecal fermentation using batch culture and analyses of Short-Chain Fatty Acids (SCFAs). The growth of *Bifidobacterium* has significantly increased from 0 (8.90±0.05 log^{10} cells/mL) and 72h (8.83±0.14 log^{10} cells/mL) for D glucose and FDWMP (8.75±0.07 log^{10} cells/mL (0h) and 8.87±0.12 log^{10} cells/mL (72h)). However, the increase in population was not significant for inulin (9.15±0.06 log10 cells/mL), FDPP (9.04±0.12 log^{10} cells/mL), and FDRMP (8.67±0.08 log^{10} cells/mL). The number of *Lactobacili* significantly increased at 6h for FDPP (9.11±0.07 log^{10} cells/mL) and 24h for FDWMP (8.88±0.07 log^{10} cells/mL) and FDRMP (8.80±0.09 log^{10} cells/mL). Acetic acid was detected in all samples, and the concentration increased in all vessels at any given time except for the FDWMP fermentation, which decreased after 0h and increased after 6h. Overall, FDWMP has increased the probiotic growth of *L. plantarum* TISTR 1465 and exhibit the highest prebiotic index. As a result, it is suggested that the FDWMP be potentially used as a healthy raw material in developing varieties of functional prebiotic food products.

Keywords: fecal samples, freeze-dried, gas production, gut microbiota

INTRODUCTION

The *Cucurbitaceae* family is one of the most genetically distinct food plants widely planted in tropical areas (Karam *et al.* 2016). Cucurbit fruits are edible, and the taste of this pericarp is usually sweet and can be grown worldwide, with over 130 genera and 800 species (Duan *et al.* 2016).

Pumpkins in Malaysia are derived from the *Cucurbita moschata* (Men *et al.* 2021). Because it benefits the lungs and spleen, *Cucurbita moschata* has been used as a traditional medicine and healthy food in China (Bergantin *et al.* 2018). Proteins, polysaccharides, para-aminobenzoic acid, and sterols are the bioactive molecules in the seeds, leaves, and flesh. Pumpkin pulp is high in polysaccharides (60−80%), has anti-tumor, anti-diabetic, and immune-stimulating properties (Men *et al.* 2021).

The winter melon (*Benincasa hispida*) is a Southeast Asian native cultivated for over 2,000 years (Karam *et al.* 2016). This cucurbit plant is also known as Kundur (Malaysia), Kushamanda (India), Dnggu (China), and Bleego (Indonesia) (Mohammad *et al.* 2019). Winter melon has an antioxidative capacity and has been shown to benefit a variety of tissues, including the brain and liver (Islam *et al.* 2021). Rock melon (*Cucumis melo L.*), is a popular summer fruit due to its sweet, juicy flesh and pleasant aroma. Rock melon is high in nutritional value, and the seeds are high in fat and protein (Adams *et al.* 2014). These cucurbit plants are among the most popular fruits and are thought to have some prebiotic properties.

Prebiotics are fermented ingredients selectively used by host microorganisms for good health (Sommer & Bäckhed 2013). In addition,

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(Received 21-07-2023; Revised 06-11-2023; Accepted 11-03-2024; Published 31-08-2024)

the consumption of prebiotics increases the population of beneficial bacteria in the gut colon and, consequently, prevents the establishment of pathogenic bacteria. Thus, it helps to prevent allergies and infections (Thursby & Juge 2017). Following ingestion, gut microbiota ferments the prebiotic constituents in the colon to produce Short-Chain Fatty Acids (SCFAs), namely acetic, propionic, and butyric acid. These acid productions improve several health physiological effects such as bowel function, regulation of lipids, mineral absorption, glucose metabolism, and decreasing the risk of colorectal cancer development (Thursby & Juge 2017).

Drying is a standard method for preserving food items over time by lowering water activity (a_w). The a_w below than 0.3 provides a longer shelf life because less free water is accessible for biochemistry activities. The aw must be kept to a minimum for living organisms to survive during manufacturing, storage, and consumption (Wang *et al.* 2020).

Previous investigations have established freeze-drying to dehydrate various fruits and vegetables, including guava, strawberries, pumpkin, tomatoes, and maple syrup (Bhatta *et al.* 2020). Several studies have shown various beneficial impacts of freeze-drying processes on the physicochemical characteristics, bioactive constituents, and antioxidant potential of fruits and some plants (Kittibunchakul *et al.* 2023; Borges *et al.* 2023). However, no study has investigated and compared the prebiotic properties of freeze-dried pumpkins, winter melons, and rockmelons. To the best of our knowledge, there is scarce information on the investigation of the cucurbit plant on prebiotic activity and in vitro fermentation by gut microbiota. The purpose of this study was to determine the effects of the freeze-drying method on nutrient composition, evaluate bacterial growth modulation of primarily beneficial bacteria using Fluorescence in Situ Hybridization (FISH), investigate the SCFAs, and calculate the Prebiotic Index (PI) to assess the prebiotic effects of selected cucurbit plants on microbial populations.

METHODS

Design, location, and time

The study was an experimental design. The research was conducted at the NFF Laboratory, Prince of Songkla University, Thailand. This study was carried out between March 2018 and May 2019. For taking fecal samples, we have received a consent from the donors which approved by the ethical committees of USM (USM/JEPeM/19030181).

Materials and tools

Pumpkin, winter melon, and rock melon were purchased from a local supermarket in the Kota Bharu district of Kelantan state, Malaysia. Their skins and seeds were removed, and the flesh was manually cut into small, thin pieces (0.5cm). The materials were frozen in a freezer (National NR-B53FE, Malaysia) at -20°C for 48h. The materials were freeze-dried for 36h in a freeze-dry (-40°C, vacuum speed 0.92 mbar, Christ-Alpha 1-4 LO, Germany). The samples were then finely ground using an electric grinder (National MX-895M, Malaysia) at low speed (1) for 10 min and sieved using a mechanical sieve (Retsch AS 200, Germany) to obtain standardized small particle powder. Each freeze-dried sample was labeled as FDPP (Freeze-Dried Pumpkin Powder), FDWMP (Freeze-Dried Winter Melon Powder), and FDRMP (Freeze-Dried Rockmelon Powder). The powder was stored in sealed airtight laboratory containers (Schott DURAN, Germany) at 4°C.

Artificial saliva, Simulated Gastric Fluid (SGF), and bile salt were purchased from Sigma-Aldrich, USA. Enzymes (alpha-amylase, pepsin, and pancreatin) were bought from Sigma-Aldrich, USA. Probes of DNA (Bif164, Lab158, Cris150, Bac303, and Eub338) used for the FISH method were purchased from Sigma-Aldrich, USA.

Procedures

Proximate composition. The AOAC (2000) method was used to calculate the proximate parameters of the FDPP, FDWMP, and FDRMP (ash, moisture, fat, protein, and total dietary fiber) including the carbohydrate.

Simulated gastrointestinal conditions of cucurbit fruits. The simulation of upper gut digestion was carried out according to the protocols of (Frank & Pace 2008) with some modifications.

Fecal sample preparation. Fresh fecal samples were provided by five healthy donors (3 women and two men, ages 25 to 35) who met the inclusion and exclusion criteria for batch culture fermentations. The storage conditions of feces were followed (AOAC 2020). The preparations of fecal slurry were followed according to Ying *et al.* (2018) with modifications.

The inclusion and exclusion criteria for subjects; included age between 20 to 60 years old, no antibiotics for at least 3 months before entering the study, no history of Gastrointestinal (GI) diseases, no consumption of products containing high levels of inulin or oligofructose (chicory, onion, banana, Jerusalem artichoke, and dragon fruit) 1 week before and during this treatment period (Liu *et al.* 2014).

Prebiotic index (PI) determination. The PI of cucurbit samples was computed based on the equation as described below:

$$PI = \left(\frac{Bif}{Total}\right) - \left(\frac{Bac}{Total}\right) + \left(\frac{Lac}{Total}\right) - \left(\frac{Clos}{Total}\right)$$

FISH analysis. The protocols for assessing bacterial population were followed according to Adeleke & Odedeji (2010).

SCFAs determination by HPLC. Short-Chain Fatty Acids (SCFAs) were analyzed by High-Performance Liquid Chromatography (HPLC) according to Alfilasari *et al.* (2021).

Data analysis

All statistical analyses were implemented using SPSS software version 27 (SPSS Inc., USA). The data were statistically analyzed for ANOVA, and mean values were assessed for statistical significance ($p<0.05$) by Duncan's multiple range test (95% Confidence Interval (CI)). The post hoc Tukey's test, at 95% CI, was assessed to differentiate between the mean values in the results proximate composition of all samples.

RESULTS AND DISCUSSION

Proximate compositions of selected cucurbit plants.

After freeze-drying, the moisture content of FDPP is significantly ($p<0.05$) lower at 7.39% than that of FDWMP and FDRMP, which are 9.83% and 9.84% (Table 1), respectively. Sun-dried pumpkin powder has a moisture level of 11.72% (Kiharason *et al.* 2017), which is higher than the FDPP produced in this investigation. High moisture content of a fresh sample reduces the quality of the stored sample due to water content, which might contribute to microbial development (Dávila *et al.* 2019). The food product's low moisture content ensures a long shelf life, minimizes perishability and boosts the food's value and shelf life (Promjiam *et al.* 2017).

The sample FDWMP had the greatest ash content, with 10.86%, compared to FDPP (8.50%) and FDRMP (5.22%), respectively. According to Kiharason *et al.* (2017), the ash level of pumpkin flour is lower than FDPP, at 5.29%. This lower value is due to the different technique of drying process performed by them as compared to our study where the cucurbit samples are underwent freeze-drying process. The samples had a considerable increase in ash composition after drying due to water removal, which increased nutrient concentration (Siti Mahirah *et al.* 2018). Furthermore, a rise in ash content during dehydration might be explained by low mineral volatility, which is unaffected by heating. A high ash content indicates a higher mineral concentration in particular food items (Ng *et al.* 2020).

In terms of other nutrients, the fat levels of FDPP, FDWMP, and FDRMP differed significantly ($p<0.05$), with 1.52%, 0.49%, and 0.22%, respectively. FDPP had the highest fat level, at 1.52%. This is advantageous because a high-fat diet has been linked to a variety of health problems. Low-fat diets may benefit from gourd fruits (Men *et al.* 2021).

The protein compositions of FDPP, FDWMP, and FDRMP differed considerably ($p<0.05$), with 8.78%, 10.51%, and 7.00%, respectively. Sample FDWMP has the greatest protein content (10.51%). The protein level of several cultivars of *Benincasa hispida* (oven-dried) ranged from 9.30% to 24.6% (Islam *et al.* 2021). These values are slightly different as compared to our study because of the different drying processes perfomed by Islam *et al.* (2021). Proteins are required in the human body to repair and replace worn-out tissues, to act as antibodies, and as a building block of cellular protein (Promjiam *et al.* 2017).

The carbohydrate contents of FDPP, FDWMP, and FDRMP differed significantly ($p<0.05$) by 73.86%, 69.61%, and 77.51%, respectively. The sample FDRMP had the greatest carbohydrate content, at 77.51%. According to Ojo *et al.* (2014) the carbohydrate content of cucumber (*Cucurbitaceae genus* was 76.13 g/100 g).

Table 1. The nutritional compositions of FDPP, FDWMP, and FDRMP

Proximate composition parameters	Samples		
	FDPP	FDWMP	FDRMP
Moisture (%)	$7.39±0.25^{j}$	$9.83±0.24^{i}$	$9.84±0.28^{i}$
Ash (%)	$8.50±0.10^{j}$	$10.86±0.13^{i}$	$5.22±0.02^{k}$
Fat (%)	$1.52±0.03^{i}$	$0.49±0.08^{j}$	$0.22±0.04^{k}$
Protein (%)	$8.78±0.05^{j}$	$10.51±0.12^{i}$	$7.00±0.17^{k}$
Total carbohydrate (%)	$73.86±0.49^{j}$	$69.61±0.24^{k}$	$77.51±0.34^{i}$
Total Dietary Fiber (TDF) (g/100 g)	$9.96±0.33^{j}$	$31.28±0.26^{i}$	$7.62±0.27^{k}$

The values are expressed as the mean and standard deviation of three determinations

$^{i-l}$Means differed significantly (p 0.05) in the same rows with different letters

FDPP: Freeze-Dried Pumpkin Powder; FDWMP: Freeze-Dried Winter Melon Powder; FDRMP: Freeze-Dried Rock Melon Powder

The TDF of the FDWMP was significantly ($p<0.05$) higher (31.28 g) than that of the FDPP (9.96 g) and the FDRMP (7.62 g). The TDF concentration of winter melon juice was quite high (27.5%) due to bioactive characteristics (Mohammad *et al.* 2019), which validated the total dietary fibre (TDF) of FDWMP found in this investigation. The TDF content of FDPP and FDRMP was significantly different ($p<0.05$), with 9.96 g and 7.62 g, respectively.

Population changes of Bifidobacterial (Bif164) analyzed by FISH technique

The populations of *Bifidobacterium* in the D glucose and FDPP increased for fermentation times up to 24h, with significant differences from the population at 0, 6, 12, and 24h ($p<0.05$), respectively (Table 2). The highest population of *Bifidobacterium* at 24h was found in FDPP with 9.25±0.06 log^{10} cells/mL. The positive control (inulin) *Bifidobacterium* was higher with significantly different ($p<0.05$) (9.35±0.05 log^{10} cells/mL) at 12h than the population in the D glucose at 24h (9.07±0.07 log^{10} cells/mL). In addition, the number of Bifidobacteria stayed elevated significantly until 12h fermentation, particularly on inulin and FDRMP, while for D glucose and FDPP, the Bifidobacterial population increased until 24h fermentation but was not significant ($p>0.05$).

During 12h fermentation, D glucose (9.04±0.04 log^{10} cells/mL), inulin (9.35±0.05 log^{10} cells/mL), FDPP (9.22±0.07 log^{10} cells/mL), FDWMP (8.80±0.09 log10 cells/mL), and FDRMP (8.96±0.19 log^{10} cells/mL) were significantly increased ($p<0.05$) in Bifidobacteria population except for FDWMP. The findings were correlated to Zhou *et al.* (2016) where, the Bifidobacteria population increased during 12h fermentation in the substrates of control, FOS, and Grape Polyphenols (GP). Also, FDPP, FDWMP, and FDRMP have shown a Bifidogenic effect (stimulate the growth of Bifidobacteria) in short time of fermentation. Among tested cucurbit samples, FDPP showed the highest growth support of Bifidobacteria. All cucurbits (FDPP, FDWMP, and FDRMP) have promoted the growth of Bifidobacteria.

Population changes of *Lactobacillus* (Lab158) analyzed by FISH technique

The *Lactobacillus* population increased after 6h of FDPP fermentation (9.11±0.07 $log1^{0}$ cell/mL), 24h of FDWMP fermentation (8.88±0.07 log^{10} cell/mL), and 12h of FDRMP fermentation (8.89±0.10 log^{10} cell/mL, Table 3). Furthermore, the number of *Lactobacillus* of D glucose was significantly increased ($p<0.05$) after 12h fermentation (8.38±0.06 log10 cell/mL) and inulin (9.40±0.04 log^{10} cell/mL), which has the highest *Lactobacillus* growth. The difference in the number of *Lactobacillus* growth among all freeze-dried samples is may because of differences in carbohydrate metabolism resulting from the metabolic and genomic diversity of *Lactobacillus* (de Andrade *et al.* 2020).

The bacteria population of *Lactobacillus* reached 8.26±0.06 (D glucose), 8.88±0.07 (FDWMP), 8.80±0.09 (FDRMP), 9.29±0.07 (inulin) and 9.23±0.11 (FDPP) log^{10} cells/mL after 24h fermentation then slightly decreased after 48h fermentation. Extending the growing period to 48h did not increase the number of viable cells in any of the starters studied (except

Table 2. Bifidobacterial faecal bacteria populations on cucurbit samples in batch culture fermentation

Bacterial count (log^{10} cells/mL); Bif164; Samples					
Time (hour)	D glucose	Inulin	FDPP	FDWMP	FDRMP
0	$8.90±0.05^{dj}$	$8.95±0.11^{dj}$	$9.03±0.08^{ci}$	$8.75±0.07^{dk}$	$8.20±0.08^{cl}$
6	$8.95±0.08^{cdj}$	$9.12±0.05^{ci}$	$9.13±0.05^{bi}$	$8.91±0.08^{abjk}$	$8.86±0.13^{ak}$
12	$9.04±0.04^{abk}$	$9.35±0.05^{ai}$	$9.22±0.07^{aj}$	$8.80±0.09^{cdm}$	$8.96±0.19^{al}$
24	$9.07±0.07^{aj}$	$9.29±0.08^{bi}$	$9.25±0.06^{ai}$	$8.84±0.06^{bck}$	$8.71±0.15^{bl}$
48	$8.99±0.05^{bcj}$	$9.30±0.04^{bi}$	$9.24±0.11^{ai}$	$8.93±0.15^{aj}$	$8.92±0.31^{aj}$
72	$8.83±0.14^{ek}$	$9.15±0.06^{ci}$	$9.04±0.12^{cj}$	$8.87±0.12^{abck}$	$8.67±0.08^{bl}$

The data present as log CFU/mL average standard deviations

$^{a-d}$Means in the same columns with different letters differed significantly ($p<0.05$)

$^{i-m}$Means in the similar rows with different letters showed significant differences ($p<0.05$)

FDPP: Freeze-Dried Pumpkin Powder; FDWMP: Freeze-Dried Winter Melon Powder; FDRMP: Freeze-Dried Rock Melon Powder

D glucose), but it did decrease cell viability (Do & Fan 2019). Scientists recommended that the minimum probiotic organism level in probiotic food products should be ranging from 10^6–10^7 CFU/mL at the time of ingestion for the best health benefits (Do & Fan 2019).

As well as having a rich dietary fiber composition, FDWMP also presented a higher protein content (10.51%) as compared to other samples (8.78% for FDPP and 7.00 for FDRMP as tabulated in Table 1). The fiber-bound polyphenols in the food matrix can reach the gastrointestinal tract and being metabolized by the gut microbiota, thus contributing to the health-related properties of dietary fiber and its impact on gut microbiota modulation (Tomas-Barberan *et al.* 2016).

Population changes of *Bacteroides* (Bac303) and *Clostridia* (Clos150) analyzed by FISH technique.

The *Bacteroides* population (Table 4) has significantly reduced ($p<0.05$) after 12h fermentation (8.75±0.06 log cell/mL) for D glucose, 9.20±0.09 for FDPP, and 8.56±0.25 log^{10} cell/mL for FDWMP and 8.98±0.03 log^{10} cell/mL for FDRMP, while 24h fermentation (9.29±0.14 log^{10} cell/mL) for inulin. After 24h of fermentation, the *Bacteroides* populations of D glucose (8.83±0.13 log^{10} cell/mL), FDPP (9.52±0.04 log^{10} cell/mL), FDWMP (8.86±0.11 log^{10} cell/mL), and FDRMP (9.16±0.03 log^{10} cell/mL) were significantly ($p<0.05$) increased. *Bacteroides* are the main bacterial genus in the large intestine that produces propionate when it ferments food fibers like oats and barley (Sreenivas & Lele 2013).

Clostridia population trends were similar to *Bacteroides* population trends. The *Clostridia* population was significantly reduced ($p<0.05$) after 6h of fermentation (7.9±20.11 log^{10} cell/mL) for D glucose, 12h of fermentation (8.80±0.09 log^{10} cell/mL) for FDPP, and 9h of fermentation (9.05±0.06 log^{10} cell/mL) for FDRMP (not significant, ($p>0.05$)). However, a significant ($p<0.05$) reduction in the *Clostridia* population on FDWMP was observed at 24h fermentation (8.53±0.20 log^{10} cell/mL) but not for inulin (9.00±0.10 log^{10} cell/mL). During the incubation hours, the *Clostridia* population of each substrate gradually decreased. This result implicates the potential of prebiotic properties among the tested samples. The decrease in both *Bacteroides* and *Clostridia* population with an increase in Bifidobacteria and *Lactobacillus* bacteria indicates the beneficial effects and prebiotic quality of the cucurbit samples. Based on our observation, it can be suggested that both fibers and cleaved sugars from polyphenolic glycosides may impede bacterial growth selectivity, decreasing *Bacteroides* and total bacteria (Zhou *et al.* 2016).

Prebiotic Index (PI)

The highest prebiotic effect was found in commercial prebiotic inulin, with 1.50, respectively. Ariestanti *et al.* (2019) reported that the PI of batch culture fermentation of inulin was slighly lower at 0.97 as compared to our finding. The PIs of D glucose, inulin, FDPP, FDWMP, and FDRMP were 1.33, 1.50, 1.75, 1.90, and 1.44, respectively. The FDWM sample had the highest PI among selected cucurbit samples, with a

Table 3. Lactobacillus fecal bacteria populations on cucurbit samples in batch culture fermentation

Bacterial count (log^{10} cells/mL); Lab158; Samples					
Time (hour)	D glucose	Inulin	FDPP	FDWMP	FDRMP
0	8.20±0.11^{cl}	9.01±0.04^{ei}	8.90±0.08^{dj}	8.72±0.09^{bk}	7.67± 0.27^{dm}
6	8.19±0.22^{ck}	9.14±0.04^{di}	9.11±0.07^{bi}	8.77±0.12^{bj}	8.72± 0.09^{bcj}
12	8.38±0.06^{al}	9.40±0.04^{ai}	8.92±0.08^{dj}	8.78±0.08^{bk}	8.89± 0.10^{aj}
24	8.26±0.06^{bcm}	9.29±0.07^{bi}	9.23±0.11^{aj}	8.88±0.07^{ak}	8.80±0.09^{abl}
48	8.38±0.05^{al}	9.26±0.07^{ci}	9.00±0.14^{cj}	8.75±0.11^{bk}	8.68± 0.09^{ck}
72	8.35±0.18^{abl}	9.13±0.03^{di}	8.92±0.12^{dj}	8.73±0.11^{bk}	8.66± 0.08^{ck}

The data are presented as log CFU/mL average standard deviations

$^{a-e}$Means differed significantly (p 0.05) in the same columns with different letters

$^{i-m}$Means differed significantly (p 0.05) in similar rows with different letters

FDPP: Freeze-Dried Pumpkin Powder; FDWMP: Freeze-Dried Winter Melon Powder; FDRMP: Freeze-Dried Rock Melon Powder

positive prebiotic effect of 1.90 and fermentation increasing the bacterial population of beneficial bacteria. A PI more than one indicates that the polysaccharide significantly affects probiotic development (Ahire *et al.* 2022). If the PI is nearly one, the evaluated carbohydrate is poor quality. In this study, all samples analysed for PI calculation yielded results of more than one, implying that the values are positive PI, equivalent to the prebiotic effect. In short, the fecal batch culture showed that FDPP, FDWMP and FDRMP showed a prebiotic potential comparable with commercial inulin.

Production of SCFAs

The AA concentration increased in all vessels at any given time except for the FDWMP fermentation, which decreased after 0h and increased after 6h (Table 5). This SCFA demonstrated a significant ($p<0.05$) difference

Table 4. Bacteroides and Clostridia faecal bacteria populations on cucurbit samples in batch culture fermentation

Cucurbit samples					
Bacterial count (log^{10} cells/mL) for Bacteroides (Bac303)					
Time (hour)	D glucose	Inulin	FDPP	FDWMP	FDRMP
0	8.74±0.07^{cl}	8.89±0.12^{dk}	9.20^{bi} ±0.09	9.11^{aj}±0.07	8.57^{dm}±0.02
6	8.82±0.11^{abm}	9.28±0.13^{bj}	9.46^{ai}±0.08	8.94^{bl}±0.18	9.07^{bk}±0.07
12	8.75±0.06^{bcl}	9.31±0.12^{abi}	9.20^{bj}±0.09	8.56^{em}±0.25	8.98^{ck}±0.03
24	8.83±0.13^{al}	9.28±0.14^{bj}	9.52$^{ai\pm}$0.04	8.86^{bcl}±0.11	9.16^{ak}±0.02
48	8.83±0.03^{al}	9.40±0.16^{aj}	9.49$^{ai\pm}$0.05	8.83^{cl}±0.12	9.16^{ak}±0.03
72	8.59±0.08^{dk}	9.06±0.19^{ci}	8.75$^{cj\pm}$0.26	8.72^{dj}±0.08	8.98^{ci}±0.03
Bacterial count (log^{10} cells/mL) for Clostridia (Clos150)					
0	8.18±0.09^{bk}	8.71±0.07^{ci}	8.77±0.13^{ci}	8.61±0.07^{dej}	8.17±0.08^{ek}
6	7.92±0.11^{cl}	8.76±0.05^{bcj}	9.14±0.08^{ai}	8.66±0.23^{cdk}	9.11±0.03^{ai}
12	8.40±0.12^{ak}	9.02±0.0^{7ai}	8.80±0.09^{cj}	9.05ai±0.08^{ai}	9.05±0.06^{bi}
24	8.24±0.17^{bl}	9.00 ±0.10^{ai}	9.00±0.10^{bi}	8.53±0.20^{ek}	8.82±0.12^{dj}
48	8.17±0.10^{bl}	8.83±0.21^{bk}	9.10±0.09^{ai}	8.93±0.14^{bj}	8.98±0.04^{cj}
72	8.14±0.23^{bk}	8.77±0.23^{bcj}	8.84±0.09^{cj}	8.75±0.05^{cj}	8.98±0.04^{ci}

The data are presented as log CFU/mL average standard deviations

$^{a-e}$Means differed significantly (p 0.05) in the same columns with different letters

$^{i-m}$Means differed significantly (p 0.05) in similar rows with different letters

FDPP: Freeze-Dried Pumpkin Powder; FDWMP: Freeze-Dried Winter Melon Powder; FDRMP: Freeze-Dried Rock Melon Powder

Table 5. Short-chain fatty acids (SCFAs) production in selected cucurbit plants

		Concentration (mM); Samples				
SCFA	Time (Hour)	D glucose	Inulin	FDPP	FDWMP	FDRMP
Acetic	0	7.32±0.20^{fi}	5.96±0.15^{fi}	9.84±6.36^{fi}	3.16±0.18^{ei}	2.59±0.0^{1fi}
	6	16.88±0.03^{ej}	26.21±0.07^{ei}	18.42±0.28^{ej}	2.47±3.49^{fk}	15.58±0.73^{ej}
	12	18.73±0.16^{dj}	33.60±4.36^{di}	23.13±0.14^{dj}	8.20±11.59^{dj}	17.08±0.34^{dj}
	24	20.13±0.07^{ck}	33.75±0.09^{ci}	27.61±0.25^{cj}	20.35±0.35^{ck}	20.24±1.15^{ck}
	48	21.62±0.06^{bl}	40.02±0.62^{bi}	28.94±0.80^{bj}	23.64±0.52^{bk}	22.79±0.27^{bkl}
	72	23.37±0.20^{ak}	53.77±2.74^{ai}	31.23±0.06^{aj}	24.19±0.54^{ak}	24.14±0.6^{1ak}
Propionic	0	3.64±0.11^{fj}	7.12±0.29^{ei}	8.06±1.06^{ei}	3.84±0.18^{fj}	3.89±0.10^{fj}
	6	5.82 ±1.39^{eij}	4.60±0.02^{fj}	5.69±0.24^{fij}	4.28±0.10^{ej}	6.73±0.50^{ei}
	12	10.06±0.07^{di}	9.16±0.18^{di}	8.59±0.11^{di}	11.10±5.02^{ai}	7.66±0.41^{di}
	24	10.21±0.06^{cj}	10.20±0.04^{cj}	11.61±0.01^{ai}	8.71±0.06^{ck}	10.13±1.01^{cj}
	48	10.84±0.06^{aj}	11.88±0.13^{bi}	10.65±0.14^{cj}	8.77±0.11^{bk}	11.03±0.71^{bij}
	72	10.73±0.56^{bj}	16.58±0.22^{ai}	10.87±0.26^{bj}	7.73±0.01^{dk}	11.38±0.13^{aj}
Butyric	0	2.28±0.64^{fi}	ND	ND	ND	ND
	6	3.15±0.17^{ei}	ND	ND	ND	ND
	12	5.07 ±0.01^{ci}	2.73±0.24^{ci}	ND	4.37±6.18^{ai}	ND
	24	5.12±0.21^{bi}	3.45±0.04^{bj}	ND	ND	ND
	48	5.59 ±0.12^{ai}	2.72±3.85^{dj}	ND	ND	ND
	72	3.86±0.60^{dj}	6.73±0.16^{ai}	1.00±0.41^{ak}	ND	ND

The values are expressed as mean standard deviations (n=2); Mean= mM concentration; ND: Not Detected

$^{a-f}$Means in the same columns with different letters showed a significant difference (p<0.05)

$^{i-m}$Means in the same rows with different letters showed a significant difference (p<0.05)

FDPP: Freeze-Dried Pumpkin Powder; FDWMP: Freeze-Dried Winter Melon Powder; FDRMP: Freeze-Dried Rock Melon Powder

in inulin (6, 12, 24, 48, and 72h), FDPP (24, 48, and 72h), FDWMP (6 and 48h), D glucose (6 and 48h), and FDRMP (48h). After 72h of AA treatment, only inulin and FDPP fermentations showed a significant (p<0.05) different, whereas D glucose, FDWMP, and FDRMP did not. Inulin fermentation in vitro produced the most AA (p>0.05) with 53.77±2.74 mM, followed by FDPP (31.23±0.06 mM), FDWMP (24.19±0.54 mM), FDRMP (24.14±0.61 mM), and D glucose (23.37±0.20 mM). *Bacteroides* produce AA, while Eubacterium-Clostridium subgroups, Fusobacterium, and Roseburia produce butyric acid (Alfilasari *et al.* 2021). Acetic acid is required for inflammation control, pathogen resistance, and tissue function (Wang *et al.* 2019).

Propionic acid resulted in a significant (p<0.05) difference in inulin during (48 and 72h), FDPP (24h), FDWMP (24, 48, and 72h), and FDRMP (24, 48, and 72h) (6 and 48h) of fermentation. At 72h, In vitro inulin fermentation produced the highest population of propionic acid (p<0.05) with 16.58±0.22 mM, followed by FDRMP (11.38±0.13 mM), FDPP (10.87±0.26 mM), D glucose (10.73±0.56 mM), and FDWMP (7.73±0.01 mM). Except for inulin, FDPP, and FDWMP, propionic concentrations increased significantly (p<0.05) with substrates D glucose and FDRMP.

On the other SCFA, butyric acid concentrations increased significantly (p<0.05) for D glucose fermentation, while inulin concentrations being the highest; however, at some points it was not detected (0 and 6h). The highest concentration of butyric acid among cucurbit plants was found in FDWMP, detected during 12h fermentation with 4.37±6.18 mM. A high butyric acid concentration is colonocytes' most crucial energy source and plays an essential role in proliferation and differentiation (Zhou *et al.* 2016). Butyric acid is helpful to humans because it delivers energy to the colonic epithelium (Rios-Covian *et al.* 2016), regulates the progression and apoptosis of epithelial and immune cells, and prevents colitis and colon cancer (Furusawa *et al.* 2013). Acetate, followed by propionate then butyrate, were the major organic acids produced in the fermentation systems which is similar to what happens in vivo during carbohydrate degradation (Wang *et al.* 2019). Our findings also show that the cucurbit samples produced the highest concentration of acetate followed by propionate and butyrate which in line with Wang *et al.* (2019).

CONCLUSION

There are variation in results on the nutritional content and the prebiotic activity score by in vitro fermentation exhibited by Freeze-Dried Pumpkin Powder (FDPP), Freeze-Dried Winter Melon Powder (FDWMP), Freeze-Dried Rock Melon Powder (FDRMP), inulin, and D glucose. Beneficial bacteria selectively ferment FDWMP and can increase acetic and propionic acid production during faecal fermentation. *Bifidobacterium* growth increased significantly between 0 and 72h for D glucose and FDWMP but not for inulin, FDPP, or FDRMP. The increase in both Bifidobacteria and *Lactobacillus* bacteria but the decrease in both *Bacteroides* and *Clostridia* indicates the beneficial effects and prebiotic quality of the cucurbit samples used in this study. The FDWMP increased the probiotic growth of *L. plantarum* TISTR 1465 and was the highest PI among selected cucurbit plants in the study. Among all cucurbits analyzed, it is proposed that the FDWMP be used as a healthy raw material in the production of a variety of functional food products.

Future works are recommended to explore the ability of food products developed with FDWMP in emeliorating the regularity and defecation behaviour among healthy individuals. It is also recommended to investigate in vitro antimicrobial activity and elucidate mechanistic action on how fiber-bound polyphenols and polysaccharides exhibit pre- and probiotic properties from cucurbit plant.

ACKNOWLEDGEMENT

We want to thank the Nutrition Laboratory School of Health Sciences, Universiti Sains Malaysia, Kelantan, Malaysia, and NFFP, Thailand, for providing the necessary facilities for this study. We also acknowledge Assoc. Prof. Santad Wichienchot, from the Prince of Songkla University, Thailand who provide a commercial inulin (Orafti®) for this study. The Malaysian Ministry of Higher Education (MOHE) supported this research with grant FRGS/1/2019/STG05/USM/01/4.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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