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Nutritional composition and prebiotic properties of freeze-dried selected cucurbit plants as potential functional food ingredients

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Abstract

Inflammatory bowel disease (IBD) is prevalent in various countries, and has been rapidly increasing in Asian countries, including Malaysia. The present work aimed to elucidate the proximate composition and prebiotic properties of each freeze-dried powder of pumpkin (FDPP), winter melon (FDWMP), and rock melon (FDRMP) from the cucurbit family. It was observed that the moisture content of FDPP was significantly the lowest at 7.39%, compared to the content in FDWMP and FDRMP at 9.83 and 9.84%, respectively. The highest protein concentration was found in FDWMP at 10.51%. The total dietary fibre of FDWMP was the highest (31.28 g) compared to FDPP (9.96 g) and FDRMP (7.62 g). FDWMP showed a potential prebiotic effect only at initial from 0 to 12 h (*Lactobacillus plantarum* TISTR 1465), and FDRMP presented a comparatively prebiotic effect by increasing its number from 6.00×10^6 to 1.01×10^6 CFU/mL within 72 h (*Bifidobacterium* BB12). The FDWMP can be used for further application as an alternative potential functional food ingredient to improve nutritive values, dietary fibres, and prebiotic properties. Further study is needed to investigate the prebiotic properties of FDWMP in food products.

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Introduction

Inflammatory bowel disease (IBD) is known as a chronic disease that is identified by the condition of remitting and relapsing inflammation in the intestine. In Malaysia, the prevalence of IBD incidence has been skyrocketing for the past two decades (0.07 to 0.69 per 100,000 people) (Mokhtar *et al.*, 2019). A long-term complication of IBD leads to the incidence of colorectal cancer (Yusof *et al.*, 2013). IBD can be minimised through the intake of non-digestible oligosaccharides (prebiotics) which stimulate the proliferation of probiotic microbiota in the gastrointestinal tract (Wong *et al.*, 2022), and resist digestive enzymes (Ji *et al.*, 2021). Besides, prebiotics have been documented to ameliorate endoscopic signs and clinical outcomes in active ulcerative colitis (UC) patients (Wong *et al.*, 2022).

Beneficial microorganisms (probiotics) assist the host by ameliorating gut preventive function, changing the microbiota composition, and modulating the immune response. IBD patients who regularly used probiotics experienced less severe pain (Mak *et al.*, 2020). Usually, there are two major factors that contribute to IBD: urbanisation and a shift towards a Western diet.

An imbalanced diet is one additional factor that may be associated with abnormal intestinal microflora and disturbances in the immune system, thus triggering the prevalence of IBD. Low consumption of dietary fibre from fruits and vegetables is one of the factors that contribute to the incidence of IBD. Contradictorily, a significant intake of dietary fibre, considered prebiotics, has been associated with reduced risks of having IBD, thus suggesting a protective effect of these polysaccharides (Reynolds *et al.*, 2019). Prebiotics exhibit gut health-protective and beneficial effects on the bowel through the metabolism of specific beneficial microorganisms (Luo *et al.*, 2021). Some natural resources, such as fruit (banana), whole grains (barley, rye, wheat), beans (soybean, peas), and vegetables (Jerusalem artichoke, tomato, chicory, onion, garlic, asparagus, beets) can be good sources of prebiotics (Davani-Davari *et al.*, 2019).

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Fruits from the Cucurbitaceae family are widely grown in tropical climates (Simpson and Morris, 2014). Cucurbit fruits such as winter melon, rock melon, watermelon, and honeydew, and the starchy cucurbits, namely gourds, pumpkins, and squashes, are fleshy and edible, and the taste of these pericarps is sweet. Cucurbits can be planted worldwide in approximately 130 genera and 800 species (Dhiman *et al.*, 2012). Among several cucurbit extracts, an active ingredient is dietary fibres, as found in pumpkins (*Cucurbita moschata*) (Fadzirul *et al*., 2018), which interact with organisms, and cause a change in biological activity. Dietary fibres can regulate the immune system by stimulating macrophages, having anti-tumour effects, decreasing inflammation, and being hypoglycaemic agents (Ji *et al*., 2021). Insoluble dietary fibres (specifically prebiotics) are a good medicine as they are regularly consumed, and unlikely to be harmless to humans (Ji *et al*., 2021).

In China, pumpkin has been consumed as a healthy food and folk medicine as they believe that it is good for the spleen and lungs (Ji *et al*., 2021). Traditional medicinal complex carbohydrates have been shown to have physiological traits, namely tumour growth prevention, wound healing, immunomodulation, and anti-hyperglycaemia effects (Salehi *et al*., 2019). Pumpkin is considered a healthy source of carotenoids, vitamins, and various health-beneficial substances. Pumpkin is reported to have anti-diabetic, anti-carcinogenic, and anti-microbial potentials (Yok *et al*., 2016). Besides, pumpkin utilised for fermentation influences the antioxidant activity, composition of bioactive compounds, number of potentially beneficial microbiota in the end products, and sensory quality of the pumpkin frozen desserts (Szydłowska *et al*., 2022).

Other commonly consumed cucurbits are winter melon (*Benincasa hispida)* which has been reported to have anti-obesity effect. Cantaloupe melons (*Cucumis melo*) are reported to contain the highest concentration of β-carotene (pro-vitamin A), a functional compound essential for vision health, and exhibit antioxidative capacity to prevent the risks associated with cardiovascular disease, cancer, and other illnesses (Aisyah *et al*., 2018). Rock melon is a commercially important crop in some countries, including Malaysia. Both *C. melo* ssp. *Agrestis* and *C. melo* ssp. are characterised by their excellent flavourings which are very rich in nutritive values and a great source of pharma-nutritional constituents for humans (Silva *et al.*, 2020). Rock melon carotenoid produces an orange-yellowish colour as well as sweet substances. The cell wall of rock melon's complex carbohydrates contains cellulose, pectin, and hemicellulose. The three bioactive nutrients in rock melon, namely zinc, lithium, and cucurbitacin-β function in fighting depression, ulcers, dandruff, preventing cancer, and stimulating the immune system (Lester, 1997).

Previous studies have indicated that increasing the intake of dietary fibre is effective in reducing cardiovascular disease, colorectal cancer, and type 2 diabetes (McRae, 2018a; 2018b). Even though various studies have been performed to explore the functionalities of cucurbit plants for their diverse therapeutic properties, the nutritive value information among them in gut health, specifically in prebiotic properties, is scarce. The purpose of the present work was, therefore, to analyse the nutritional composition and potential prebiotic properties of each freeze-dried powder derived from pumpkin, winter melon, and rock melon members of the cucurbit family.

Materials and methods

Chemicals

Chemicals of analytical grade were procured from Merck Sdn. Bhd. (Selangor). Celite® was acid-washed, and enzymes (alpha-amylase, protease, and amyloglucosidase) were purchased from Sigma-Aldrich, USA. The GasPak EZ anaerobic pouch system (Becton Dickinson and Company, USA) and media Man Rogosa and Sharpe (MRS) were used. A commercial inulin in white powder form with the trade name of Orafti® was provided by Assoc. Prof. Dr. Santad Wichienchot.

Kjeldahl Se tablets and $NaOH_2$ pellets were purchased from Merck Sdn. Bhd., Selangor, Malaysia. Petroleum ether and acetone were purchased from Bendosen Laboratory Chemicals, Malaysia. Sulphuric acid, boric acid, and ethanol absolute denatured were purchased from HmbG (Orioner Hightech Sdn. Bhd., Malaysia). Enzymes (α-amylase, protease, and amyloglucosidase) were purchased from Sigma-Aldrich, USA.

Sample preparation of selected cucurbit plants

The study was conducted at the Nutrition Laboratory, School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan and Nutraceutical and Functional Food Research and

Development Centre, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Selected cucurbits, namely pumpkin, winter melon, and rock melon, were purchased from a local supermarket located in Kota Bharu district, Kelantan State, Malaysia. The samples of cucurbit plants were prepared based on the study by Duarte *et al*. (2017) with some modifications. The cucurbit plants were carefully washed with distilled water, and manually peeled using a utility knife. Their skins and seeds were removed, while the flesh was manually cut into small and thin pieces (approximately 0.5 cm thick). The materials were then frozen in a freezer (Refrigerator National NR-B53FE, Malaysia) at -20°C for 48 h.

Subsequently, the materials were freeze-dried using a freeze-dryer (Christ-Alpha 1-4 LO, Germany) for 36 h. The temperature used was -40 to -50°C, and the vacuum speed was 0.92 mbar. The samples were then finely ground using an electric grinder (National MX-895M, Malaysia) for 10 min, and sieved using a mechanical sieve (Retsch Sieve Shaker AS 200, Germany) to get 150 µM particle size, thus obtaining standardised small particle powder. As for storage prior to further analyses, each freeze-dried (FD) of the samples was labelled as FDPP, FDWMP, and FDRMP, representing pumpkin, winter melon, and rock melon, respectively. Subsequently, the powder was properly kept in sealed airtight laboratory containers (Schott DURAN, Germany) at 4°C to prevent dehydration.

Proximate composition

The composition of moisture, ash, protein, fat, and total dietary fibre for FDPP, FDWMP, and FDRMP were determined according to the AOAC (2020). To begin the determination of the moisture content, the empty dish with a cover was dried in the oven at 105°C for 3 h. After 3 h, the dish was transferred into a desiccator to let cool, and was weighed. Exactly 5 g of the sample was weighed into the uncovered dish before placing the dish in the oven overnight at 105°C. After drying, the dish containing the sample was transferred into the desiccator to let it cool. The moisture content was calculated using Eq. 1:

$$\text{Moisture (\%)} = \frac{\text{Loss of weight in (g) of the sample}}{\text{Weight in (g) of the sample taken}} \text{ x } 100 \quad \text{(Eq. 1)}$$

The total ash was measured using an air oven with slight modifications. The crucible was placed in the air oven at 105°C for 3 h to ensure that impurities were burned off, and cooled in a desiccator until it had attained room temperature. The crucible was weighed, and 0.5 g of the homogenised sample was placed in it. Then, the dried sample was charred on an electric coil heating rack until it ceased smoking. The crucible was placed in the cold muffle furnace, and heated at 550°C until whitish or greyish ash was obtained. The crucible was removed and cooled down in the desiccator until it reached room temperature. After cooling down, the crucible with the ash content was weighed. Then, it was replaced with a muffle and continued heating until the weight was constant. The ash content was calculated using Eq. 2:

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \text{ x } 100 \quad \text{(Eq. 2)}$$

The protein content was determined following the Kjeldahl method (AOAC, 2020). All samples were digested with a strong acid (H_2SO_4) before proceeding with the titration technique. During the distillation, the liberated ammonia was collected in a receiver solution, and titrated with an acid solution. The amount of protein present in each sample was then calculated using the nitrogen conversion factor of 6.25.

The crude fat content was measured using semi-continuous extraction (Soxhlet method). About 3 g of the homogenised sample was weighed onto a weighing boat, and transferred to a dry thimble. Exactly 80 mL of petroleum ether was poured into the collecting vessel, which was dried and weighed first, and attached to the heating mantle for fat extraction. After 30 min, the extract-collecting vessel was dried in an oven for 45 min at a temperature high enough to completely evaporate solvent residues. After drying, the collecting vessel desiccator was cooled, and the bottle and its dried content were reweighed. The crude fat content was calculated using Eq. 3:

$$\text{Fat (\%)} = \frac{Weight\ of\ fat\ (g)}{Weight\ of\ sample\ (g)} \text{ x } 100 \quad \text{(Eq. 3)}$$

The carbohydrate content was determined according to Nordiana *et al.* (2019) by calculating the percent remaining after all the other components were measured, using Eq. 4:

Total carbohydrate = 100 - (% moisture + % ash + % fat + % protein) (Eq. 4)

For total dietary fibres (TDF), the enzymatic gravimetric method was used following the AOAC (2020). The defatted sample was cooked at 100°C with heat-stable α-amylase to give gelatinisation, hydrolysis, and depolymerisation of starch. Then it was incubated at 60°C with protease and amyloglucosidase enzymes. Ethanol was then added to precipitate the soluble fibre, and the residue was washed with ethanol and acetone before being dried and weighed. One portion of the sample was analysed for protein and ash. TDF was calculated as the weight of the residue minus the weight of the protein, and ash was reported as a percentage of the original sample weight.

Evaluations of prebiotic property potential

Preparations of MRS

The medium used to support the proliferation of *Lactobacillus* and *Bifidobacterium* was based on De Man *et al*. (1960). The preparations of de Man, Rogosa, and Sharpe (MRS) media with a growth control of 2% D-glucose, a positive control of 2% inulin, and 2% from each FDPP, FDWMP, and FDRMP were adapted from de Albuquerque *et al*. (2020) with some modifications. The formula contains 5 g of yeast extract, 10 g of beef extract, 10 g of peptone, 20 g of D-glucose, 1 mL of Tween 80, 2 g of ammonium citrate ($C_6H_{11}NO_7$), 5 g of sodium acetate ($Na_3C_6H_5O_7$), 0.1 g of $MgSO_4$, 2 g of K_2HPO_4, and 0.05 g of $MnSO_4.H_2O$.

All ingredients were mixed and dissolved well using a hotplate with frequent agitation until the mixture completely dissolved. The colour of the culture medium was amber and slightly opalescent. The temperature to store prepared culture media was 2 - 8°C. Subsequently, the preparation of MRS media with agar was done by adding 12 g of bacteriological agar and 0.2 g of bromocresol purple and letting it dissolve. The same formula was used for *B. lactis* media, but 0.05% L-cysteine-hydrochloride was added. The MRS media and MRS agar were then autoclaved for 15 min at 121°C.

Probiotic microorganisms and culture conditions

Both *L. plantarum* TISTR 1465 and *B. animalis* subsp. *lactis* BB12 probiotic strains were used to assess the potential prebiotic potential of FDPP, FDWMP, and FDRMP. *L. plantarum* TISTR 1465 was purchased from the Thailand Institute of Scientific and Technological Research, and *B. animalis* subsp. *lactis* BB12 was provided by Assoc. Prof. Dr. Santad Wichienchot (Nutraceuticals and Functional Food Laboratory - NFF, Prince of Songkhla University, Hat Yai, Thailand). *L. plantarum* TISTR 1465 was stored at -20°C in MRS media, while *B. animalis* subsp. *lactis* BB12 was kept in a freezer at -20°C until further use (Bersaneti *et al*., 2019).

The protocols for preparing probiotics were adapted from de Albuquerque *et al*. (2020) with some modifications. Exactly 500 µL of bacterial stock solution *L. plantarum* TISTR 1465 was centrifuged (Microliter centrifuges MIKRO 22 R, Hettich, France) at 8,000 rpm, 4°C, for 15 min, and then the supernatant was removed. The sediment (0.5 mL) of lyophilised culture was transferred to a test tube containing MRS broth, autoclaved for 15 min at 121°C, and incubated in an incubator (NFF Laboratory) at 37°C for 24 h. Next, MRS broth was centrifuged at 8,000 rpm at 4°C for 15 min, and the supernatant was discarded. The sediment was then mixed with 1 mL of sterilised PBS before being centrifuged at 8,000 rpm for 15 min at 4°C, and this step was repeated three times to obtain a starter culture (inoculum) of 1.38×10^6 CFU/mL.

Exactly 0.1 g of freeze-dehydrated powder of *B. animalis* subsp. *lactis* BB12 was dissolved in a test tube containing MRS media, and autoclaved at 121°C for 15 min. Then, 0.5 mL of paraffin liquid was added to the media to lower the redox potential (Sousa *et al*., 2015), and incubated for 48 h at 37°C. Next, MRS broth was centrifuged at 8,000 rpm at 4°C for 15 min, and the supernatant was then discarded. The sediment was then added to 1 mL of sterilised PBS before being centrifuged at 8,000 rpm at 4°C for 15 min, and this step was repeated three times to obtain a starter culture (inoculum) of 1.38×10^6 CFU/mL. The strains were then routinely sub-cultured (every 24 h) in MRS broth, and incubated at 37°C.

Prebiotic evaluations

The microbial plate count method was used for prebiotic evaluations. The protocols for prebiotic evaluation were adapted from Mariano *et al*. (2020) with some modifications. The quantification of probiotic *L. plantarum* TISTR 1465 was conducted using the drop plate technique following Herigstad *et al*. (2001) with some modifications. MRS media with agar was sterilised and cooled to 45 - 50°C before

being dispensed into sterilised Petri dishes. Each Petri dish was then labelled with colony numbers ($^{-3}$, $^{-4}$, $^{-5}$, and $^{-6}$), and separated into four quadrants. Tenfold serial dilutions were performed according to Gorsuch *et al.* (2019) with minor modifications. From 5 mL of each sample (MRS media), 100 µL was taken and added to 900 µL of sterilised NaCI (0.85%), and tenfold serial dilutions were gently diluted using a pipette for 1 min. For counting, each surface quadrant of MRS agar was dropped with four spots of inoculum, and each spot was 10 µL. The Petri dishes were placed in an anaerobic jar (NFF Laboratory) with GasPak EZ anaerobic to generate anaerobic condition, and incubated for 48 h at 37°C. The samples were taken at 0, 12, 24, and 48 h. Data analysis was performed with only plates comprising 25 to 250 colonies (Gorsuch *et al.*, 2019). The number of colonies grown on the surface of the medium was counted and reported as log CFU/mL. The prebiotic evaluations on FDPP, FDWMP, and FDRMP were tested in triplicate.

The quantification of probiotic *B. animalis* subsp. *lactis* BB12 was performed by the pour plate technique, modified from Boczek *et al*. (2014). After MRS agar was sterilised, it was cooled to 45 - 50°C. Each petri dish was labelled with different colony numbers ($^{-3}$, $^{-4}$, $^{-5}$, and $^{-6}$). From 5 mL of each sample (MRS media), 150 µL was taken and added to 1,350 µL of sterilised NaCI (0.85%), and serial dilutions were performed. To count, sterilised Petri dishes were inoculated with 1 mL of inoculum, and sterilised MRS agar was poured, gently swirled (2 min), and placed in an anaerobic jar with GasPak EZ anaerobic to create anaerobic condition, and then incubated for 72 h at 37°C. The samples were taken at 0, 12, 24, 48, and 72 h (Fachin *et al.*, 2008). Data analysis was performed with only plates comprising 25 to 250 colonies. The number of colonies grown on the surface of the medium was counted and reported as log CFU/mL. Each sample was analysed in triplicate.

Statistical analyses

All statistical analyses were conducted using version 27 of the SPSS software (SPSS Inc. Chicago, IL, USA). The experimental data were analysed using ANOVA, and the statistical significance of the mean values ($p < 0.05$) was established using the *post hoc* test. Tukey's test, with a confidence interval of 95&, was used to distinguish between the mean values of the results of the approximate composition of FDPP, FDWMP, and FDRMP. To determine the significant differences between samples, Duncan's new multiple range test with a confidence interval of 95% was utilised.

Results and discussion

Proximate composition

Among all samples, the moisture content of FDPP was significantly ($p < 0.05$) the lowest at 7.39%, compared to FDWMP and FDRMP at 9.83 and 9.84%, respectively (Table 1). The moisture content of FDPP after being freeze-dried was aligned with the study of the leaves of *Ocimum basilicum*, which was also significantly lower at 7.99% (Siti Mahirah *et al*., 2018). Moisture is the most important parameter necessary to be concerned about when it comes to storage (Norawanis *et al.*, 2014), because high water content contributes to microbial growth (Sospedra *et al*., 2010). Low moisture content of food product guarantees a longer shelf life (Ojo *et al*., 2014), decreases perishability, and improves the food's value (Agoreyo *et al*., 2011).

Table 1. Proximate composition of FDPP, FDWMP, and FDRMP.

Proximate composition (%)	Sample		
	FDPP	**FDWMP**	**FDRMP**
Moisture	7.39 ± 0.25^{b}	9.83 ± 0.24^{a}	9.84 ± 0.28^{a}
Ash	8.50 ± 0.10^{b}	10.86 ± 0.13^{a}	5.22 ± 0.02^{c}
Fat	1.52 ± 0.03^{a}	0.49 ± 0.08^{b}	0.22 ± 0.04^{c}
Protein	8.78 ± 0.05^{b}	10.51 ± 0.12^{a}	7.00 ± 0.17^{c}
Total carbohydrate	73.86 ± 0.49^{b}	69.61 ± 0.24^{c}	77.51 ± 0.34^{a}
Total dietary fibre	9.96 ± 0.33^{b}	31.28 ± 0.26^{a}	7.62 ± 0.27^{c}

Values are mean ± standard deviation of triplicate determinations. Means in the same row followed by different lowercase superscripts are significantly different ($p < 0.05$). FDPP: freeze-dried pumpkin powder; FDWMP: freeze-dried winter melon powder; and FDRMP: freeze-dried rock melon powder.

The highest ash content was found to be significantly different ($p < 0.05$) in FDWMP at 10.86%, compared to FDPP (8.50%) and FDRMP (5.22%), respectively. The ash content of FDWMP after being freeze-dried was comparable with the ash content of *O. basilicum* leaves, as reported by Siti Mahirah *et al.* (2018) at 12.83%. Due to water removal, the samples exhibited a large increment in ash after drying, thus increasing nutrient content (Morris *et al*., 2004). In addition, the increase in ash composition after drying may also be clarified by small mineral volatility, which is not damaged by thermal treatment. High ash content indicates a higher concentration of minerals in certain food items (Agoreyo *et al*., 2011).

FDPP, FDWMP, and FDRMP recorded fat content significantly ($p < 0.05$) with values of 1.52, 0.49, and 0.22%, respectively. The highest fat content was found in FDPP at 1.52%. Low-fat contents in pumpkin and winter melon were also reported to be similar at 0.02% (De Escalada Pla *et al.*, 2005). In general, fruits are low in fat, and have been recommended as part of a diet that reduces body weight. This is beneficial because eating a high-fat diet has been linked to an increased risks of developing non-communicable diseases. The very low fat content in cucurbit fruits can be ideal for individuals who consume a low-fat diet (Bello *et al*., 2014).

The protein contents of FDPP, FDWMP, and FDRMP showed a significant ($p < 0.05$) difference at 8.78, 10.51, and 7%, respectively. The highest protein concentration was found in FDWMP at 10.51%. The protein concentration of cucurbit fruits after being freeze-dried in the present work was found to be higher than in a previous study reported by Adebayo *et al.* (2013), with the protein content of fresh pumpkin using oven drying being 3.07%. In the human body, proteins are needed to repair and replace worn-out tissues, act as antibodies, as the key sources of various amino acids, and as the building blocks of cellular protein (Adeleke and Odedeji, 2010).

The carbohydrate contents of FDPP, FDWMP, and FDRMP were significantly ($p < 0.05$) different at 73.86, 69.61, and 77.51%, respectively. In a previous study, the carbohydrate content of cucumber (another Cucurbit) was 76.13 g/100 g (Hidayat *et al*., 2021). On the other nutrients, total dietary fibre of FDWMP was significantly ($p < 0.05$) highest (31.28 g) compared to FDPP (9.96 g) and FDRMP (7.62 g). Sun *et al*. (2017) reported that the dietary fibre content of winter melon juice was reasonably high (27.5%) due to their bioactive properties, which supported the finding of total dietary fibre in FDWMP observed in the present work. The total dietary fibre contents of FDPP and FDRMP were significantly ($p < 0.05$) different at 9.96 and 7.62 g, respectively. The results of these cucurbit fruits after being freeze-dried were compatible with the result of crude fibre basil at 10.0% (Agunbiade *et al*., 2015). Dietary fibre content is an important bioactive compound with functional properties such as nutraceuticals that improve human physiological performance by preventing or treating illnesses and disorders (Wildman, 2001).

Evaluations of prebiotic properties

The growth of *L. plantarum* TISTR 1465 on FDPP was promoted from 2.50×10^7 to 1.70×10^8 log CFU/mL; however, at 24 and 48 h, it decreased to 3.71×10^7 and 1.40×10^7 log CFU/mL (Table 2). Thus, FDPP was not capable of promoting the

Table 2. Growth-promoting effect of probiotic *L. plantarum* TISTR 1465 in various carbon sources.

Incubation time (h)	Sample				
	D glucose	**Inulin**	**FDPP**	**FDWMP**	**FDRMP**
	Microbial counts (CFU/mL)				
0	$1.46 \times 10^7 \pm 0.93^{dm}$	$1.96 \times 10^8 \pm 0.05^{bl}$	$2.50 \times 10^7 \pm 0.61^{bk}$	$5.88 \times 10^6 \pm 0.07^{ai}$	$3.67 \times 10^7 \pm 0.82^{dj}$
12	$1.66 \times 10^9 \pm 1.19^{cl}$	$1.60 \times 10^9 \pm 0.08^{dm}$	$1.70 \times 10^8 \pm 0.08^{ck}$	$2.43 \times 10^9 \pm 0.05^{cj}$	$9.08 \times 10^7 \pm 0.51^{ai}$
24	$3.16 \times 10^9 \pm 0.07^{bk}$	$1.76 \times 10^9 \pm 0.04^{cl}$	$3.71 \times 10^7 \pm 0.18^{aj}$	$1.13 \times 10^9 \pm 0.30^{dm}$	$3.80 \times 10^8 \pm 0.12^{ci}$
48	$3.75 \times 10^9 \pm 0.23^{ak}$	$9.43 \times 10^8 \pm 0.15^{ai}$	$1.40 \times 10^7 \pm 0.12^{dm}$	$3.45 \times 10^7 \pm 0.55^{bi}$	$3.93 \times 10^9 \pm 0.53^{bj}$

Values are mean ± standard deviation of triplicate determinations. ($^{a-d}$) Means in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$). ($^{i-m}$) Means in the same row followed by different lowercase superscripts are significantly different ($p < 0.05$). FDPP: freeze-dried pumpkin powder; FDWMP: freeze-dried winter melon powder; and FDRMP: freeze-dried rock melon powder.

proliferation of *L. plantarum* TISTR 1465. On the other hand, the growth of *L. plantarum* TISTR 1465 was greatly promoted by FDWMP. Besides, the growth of *L. plantarum* TISTR 1465 on FDWMP produced significantly ($p < 0.05$), from 5.88×10^6 to 3.45×10^7 log CFU/mL. The growth of *L. plantarum* TISTR 1465 on FDRMP was not improved, and the log number did not increase from 0 h (3.67×10^7) to 12 h (9.08×10^7) log CFU/mL, even though it increased at 24 and 48 h from 3.80×10^8 and 3.93×10^9 log CFU/mL. The growth of *L. plantarum* TISTR 1465 on D-glucose increased from 1.46×10^7 to 3.75×10^9 log CFU/mL. However, D-glucose has not been recognised as a prebiotic because it does not selectively stimulate the growth of probiotic strains. The growth of *L. plantarum* TISTR 1465 on the commercial inulin was also significantly ($p < 0.05$) be promoted from 1.96×10^8 to 9.43×10^8 log CFU/mL.

In vitro studies indicated that the count of lactobacilli was higher when they were cultured in the oligosaccharides-enriched medium than those in the control medium (Sreenivas and Lele, 2013). Pumpkin pulp, an agricultural residual or by-product is suggested as a potential resource to produce prebiotics, which may be utilised as a functional ingredient in the development of either health foods or supplement products (Du *et al.*, 2011). The present work corroborated Sreenivas and Lele (2013) who documented that winter melon possessed potential prebiotic properties. In *in vitro* fermentation, winter melon was shown to support the proliferation of all pure cultures, as well as mixed cultures for all microbial strains that were used in that study.

The present work also corroborated Nattaporn and Pranee (2011) who conducted a study of the effect of commercial pectinase, Pectinex Ultra SP-L on pharma-nutritional antioxidative constituents of the placenta and flesh, and the placenta of fully ripened cantaloupe (Sun Lady). The results found that the counts of *B. lactis* BB12 and *L. acidophilus* La5 grown in inulin and the flesh of cantaloupe were higher than those of other types of vegetables. Besides, other studies revealed that the prebiotic activity of sorghum flour in growth-promoting *L. plantarum* increased from 6.78 to 9.44 log CFU/mL, with the greatest value being 11.17 log CFU/mL after 28 h of fermentation (Pranoto *et al.*, 2013).

Inulin stimulated the growth of *L. plantarum* by increasing its numbers from 0 to 48 h. Hence, the result of prebiotic inulin has stimulated the good growth of the probiotics used in the present work. In a similar study, the inulin content as a carbon source stimulated a significant growth of *L. delbrueckii* BCC13296 within 48 h of incubation, from 9.14×10^7 to 8.24×10^8 log CFU/mL (Wichienchot *et al.*, 2010). Two probiotic strains were cultivated using oligosaccharides isolated from dragon fruit's flesh as an energy source. Within 48 h of fermentation, the oligosaccharides increased the number of *L. delbrueckii* BCC13296 cells from 9.02×10^7 to 6.17×10^9 cell/mL, indicating that they stimulated this probiotic growth (Wichienchot *et al.*, 2010).

The results of prebiotic evaluations of D-glucose, inulin, FDPP, FDWMP, and FDRMP in promoting growth of probiotic *B. animalis* subsp. *lactis* BB12 are shown in Table 3. The strain of *B. lactis* BB12 was cultivated in the MRS broth at 37°C. All samples (D-glucose, inulin, FDPP, FDWMP, and

Table 3. Growth-promoting effect of probiotic *B. animalis* subsp. *lactis* BB12 in various carbon sources.

Incubation time (h)	Sample				
	D glucose	Inulin	FDPP	FDWMP	FDRMP
	Microbial count (CFU/mL)				
0	$2.48 \times 10^7 \pm 0.01^{ck}$	$2.64 \times 10^7 \pm 0.01^{bj}$	$1.24 \times 10^7 \pm 0.01^{dm}$	$1.53 \times 10^7 \pm 0.01^{di}$	$6.00 \times 10^6 \pm 0.02^{bi}$
12	$9.50 \times 10^6 \pm 0.01^{ai}$	$7.56 \times 10^7 \pm 0.02^{aj}$	$1.13 \times 10^7 \pm 0.01^{ef}$	$5.06 \times 10^6 \pm 0.01^{ak}$	$1.14 \times 10^7 \pm 0.02^{di}$
24	$1.96 \times 10^6 \pm 0.01^{ei}$	$2.44 \times 10^6 \pm 0.01^{dj}$	$2.09 \times 10^6 \pm 0.01^{ck}$	$1.44 \times 10^6 \pm 0.01^{em}$	$9.26 \times 10^5 \pm 0.01^{ai}$
48	$2.10 \times 10^7 \pm 0.03^{di}$	$2.49 \times 10^6 \pm 0.01^{cj}$	$2.84 \times 10^6 \pm 0.01^{ai}$	$2.05 \times 10^6 \pm 0.01^{cm}$	$2.43 \times 10^6 \pm 0.01^{ck}$
72	$2.67 \times 10^6 \pm 0.01^{bi}$	$2.16 \times 10^6 \pm 0.01^{el}$	$2.36 \times 10^6 \pm 0.01^{bj}$	$2.27 \times 10^6 \pm 0.01^{bk}$	$20.01 \times 10^6 \pm 0.02^{em}$

Values are mean ± standard deviation of triplicate determinations. ($^{a-e}$) Means in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$). ($^{i-m}$) Means in the same row followed by different lowercase superscripts are significantly different ($p < 0.05$). FDPP: freeze-dried pumpkin powder; FDWMP: freeze-dried winter melon powder; and FDRMP: freeze-dried rock melon powder.

FDRMP) showed significant difference ($p < 0.05$) in the growth of *B. lactis* BB12 during 0, 24, 48, and 72 h of incubation. This result indicated that all freeze-dried substrates, including inulin, were good carbon sources for the growth of *B. lactis* BB12.

The growth of *B. animalis* BB12 at 0 h of incubation for FDRMP was significantly ($p < 0.05$) the highest at 6.00×10^6 log CFU/mL, compared to growth in D glucose, inulin, FDPP, and FDWMP at 2.48×10^7, 2.64×10^7, 1.24×10^7, and 1.53×10^7 log CFU/mL, respectively. Similar to this experiment, Sousa *et al*. (2015) also studied the prebiotic characteristics of yacon (*Smallanthus sonchifolius*) powder on the growth of probiotic *B. animalis* B94. The finding showed that in a medium containing MRS-glucose, the probiotic consumed the glucose during the first 8 h of fermentation.

In the present work, it was observed that the growth of *B. animalis* BB12 at 12 h incubation with D-glucose was significantly ($p < 0.05$) higher at 9.50×10^6 log CFU/mL compared to inulin and FDWMP at 7.56×10^7 and 5.06×10^6 log CFU/mL, respectively. Despite the fact that glucose is a typical carbon source for lactic acid bacteria (LAB) growth, their capacity to breakdown a variety of carbohydrates is well documented and strain-dependent (Hayek and Ibrahim, 2013), as seen in our experiments. A comparative study on prebiotic effect (*in vivo*) among male Wistar rats fed using commercial and developed mousse samples (the main ingredients were whey protein hydrolysates and pumpkin pectin), and the negative control group (only commercial mousse) had been conducted, and the findings showed that the bacterial population of *Bifidobacterium* spp. had increased by 3.7 times over the latter group (Agarkova *et al*., 2019).

At 24 h, the growth of *B. animalis* BB12 incubation for FDRMP was significantly ($p < 0.05$) the highest at 9.26×10^5 log CFU/mL, compared to growth in D glucose, inulin, FDPP, and FDWMP at 1.96×10^6, 2.44×10^6, 2.09×10^6, and 1.44×10^6 log CFU/mL, respectively. This result was in line with a previous study that focused on probiotic growth using oligosaccharides isolated from the flesh of dragon fruit (Pitaya). It was found that the oligosaccharide extracts selectively stimulated the proliferation of probiotic bacteria (*B. bifidum* NCIMB 702715), which grew insignificantly from 1.70×10^8 log CFU/mL at 0 h to 2.51×10^9 log CFU/mL at 24 h (Wichienchot *et al*., 2010).

The growth of *B. animalis* BB12 at 48 h incubation for FDPP was significantly ($p < 0.05$) the highest at 2.84×10^6 log CFU/ml compared with D-glucose, inulin, FDWMP, and FDRMP at 2.10×10^7, 2.49×10^6, 2.05×10^6, and 2.43×10^6 log CFU/ml, respectively. Meanwhile, the growth of *B. animalis* BB12 at 72 h of incubation for sample D-glucose was significantly ($p < 0.05$) the highest at 2.67×10^6 log CFU/mL compared with inulin, FDPP, FDWMP, and FDRMP at 2.16×10^6, 2.36×10^6, 2.27×10^6, and 1.01×10^6 log CFU/mL, respectively. These results agreed with Shah (2000) who reported that *B. animalis* ssp. *lactis* LAFTI® B94 was found to use both fructose and glucose substrates.

The present work also revealed that the microbial counts of D-glucose, inulin, FDPP, and FDWMP could not stimulate the growth of *B. animalis* subsp. *lactis* BB12 by decreasing its numbers from 0 to 72 h. On the contrary, FDRMP was able to stimulate the growth of probiotic *B. animalis* subsp. *lactis* by increasing their numbers from 0 to 72 h. In line with this finding, *B. animalis* subsp. *lactis* BB12 was reported to be able to grow in milk. Bifidobacteria are obligate and partially facultative anaerobes; thus, the bacteria can survive, and they are found in the final products, for instance, yoghurt. During food production and storage, the occurrence of oxygen causes the number of bacterial populations in the final products to drop (Talwalkar and Kailasapathy, 2004).

Conclusion

The freeze-drying method was effective in producing the nutritive values of some selected cucurbit plants, especially winter melon powder. Among all cucurbit fruits analysed, winter melon powder had the potential to be used by the food industry as an alternative ingredient for the formulation of functional food products due to its high total dietary fibre content and other essential nutrients. FDWMP substrate exhibited a great prebiotic effect only at initial from 0 to 12 h (*Lactobacillus plantarum* TISTR 1465), and FDRMP presented a greater prebiotic effect by increasing its number from 6.00×10^6 to 1.01×10^6 log CFU/mL within 72 h (*Bifidobacterium* BB12). In conclusion, the FDWMP is recommended to be utilised as a potential natural functional ingredient for improving nutritive values and prebiotic properties in various food products.

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