

ISOLATION AND CHARACTERIZATION OF OLIGOSACCHARIDES COMPOSITION IN ORGANICALLY GROWN RED PITAYA, WHITE PITAYA AND PAPAYA

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ABSTRACT

Objective: the objectives of this study are to isolate and characterize oligosaccharide composition in the flesh and peel of red pitaya, white pitaya and papaya using high performance liquid chromatography analysis (HPLC).

Methods: the oligosaccharide composition in both flesh and peel of red pitaya, white pitaya and papaya was determined and quantified by comparing peak areas of sugar samples to those of the standard solutions (Sigma Aldrich, USA).

Results: the results of the present study revealed both flesh and peel of red pitaya were significantly higher in dry matter as compared with white pitaya and papaya. The high total soluble solid content of the red pitaya flesh was reflected in the amount of glucose, sucrose and fructose which were significant higher than the amount in white pitaya and papaya flesh. The composition of raffinose and stachyose in red pitaya flesh was found to be significantly higher than white pitaya flesh and papaya flesh. This study clearly showed that red pitaya flesh has a significantly higher composition of maltotriose, maltotetraose and maltopentaose contents as compared to white pitaya flesh and papaya flesh. Therefore, comparatively the composition of maltotriose, maltotetraose and maltopentaose in the fruit's flesh is significantly higher in value than the peel of the fruit. The flesh of red pitaya was found to have reasonably high proportions of prebiotic oligosaccharides as compared to white pitaya and papaya.

Conclusion: the red pitaya fruit may represent a rich source of prebiotic oligosaccharides. It should be regarded as a valuable new source of prebiotic oligosaccharides with the potential of being an economic value-added ingredient to be used as a substrate for the development of functional foods to assist in the prevention of chronic diseases.

Keyword: Prebiotic oligosaccharides, Red pitaya and Functional properties

INTRODUCTION

The human gut is a natural habitat for a large and dynamic bacterial community, which is referred to as the 'intestinal microbiota'. Most bacteria are benign; however certain gut species are pathogenic and may be involved in the onset of acute and chronic disorders. Bifidobacteria and lacto-bacilli are thought to be antipathogenic and beneficial to humans [1]. The microbiota in the large intestine of humans comprises about 95% of the total cells in the body, representing 10^{12} cells/g in dry weight content. Through the activities of the resident microbiota, the colon plays a major role in host nutrition and welfare [1].

According to Ventura and Zink [2], there is much interest in increasing the numbers and activities of beneficial bacteria in the large gut, preferably at the expense of harmful bacteria. This can only be achieved by consumption of live microbial supplements, such as fermented dairy products or frozen dried cultures. Thus, because of the viability of live bacteria (probiotics) in food products and the variability during transit through the gastrointestinal (GI) tract, the concept of prebiotics has been developed and requires the selective growth of indigenous gut bacteria. Prebiotics are indigestible food ingredients that beneficially affect the host by selectively stimulating growth and/or activity of one or a number of health-promoting colon bacteria [3].

The prebiotic effect has been attributed to many food components, sometimes without due consideration to the criteria required. In particular, many oligosaccharides and polysaccharides have been claimed to have prebiotic activity, but not all dietary carbohydrates are prebiotic. Gibson and Rastall [4] suggested three criteria that need to be fulfilled for a food ingredient to be designated a prebiotic. First, substrate need not be hydrolyzed or absorbed in the stomach or small intestine. For humans, the best way to demonstrate non-digestibility is with ileostomised volunteers [5]. Second, the ingredient must be fermented in the GI tract. This can best be shown by means of a fermentation test *in-vitro*. Third, there must be selectivity in the stimulation of intestinal bacteria and of metabolic

activity. It must be selective and be beneficial to the bacteria, such as the bifidobacteria, in the gut and fermentation substrate should induce beneficial effects of luminal/systemic in its host [6].

By definition, a prebiotic substrate is not available to all bacterial species that inhabit the GI tract. In particular, it must be readily available to some groups of lactobacilli and bifidobacteria are considered as indicator organisms. These organisms are beneficial for the health of the intestine but less available to potentially pathogenic bacteria such as toxin-producing *Clostridia*, proteolytic bacteroides and toxogenic *Escherichia coli*. Any dietary component that reaches the colon intact is a potential prebiotic; however, the prebiotic property has been demonstrated adequately for only a few food ingredients.

According to Gibson and Rastall [4] only the inulin-type fructans, galacto-oligosaccharides and lactulose are proven as prebiotics. Since a number of oligosaccharides naturally occur in certain fruits and vegetables, there is a scope to separate them from their innate characteristics and use them as prebiotics and prebiotic production, to be incorporated in functional foods and nutraceuticals by the food industry. Currently, the information on oligosaccharides content and its consumption among populations around the world are lacking [6]. Thus, this study was conducted to determine oligosaccharide composition in red pitaya, white pitaya and papaya.

MATERIALS AND METHODS

Sample Extraction Procedure

The method by Xiaoli *et al.* [8] has been used in the extraction process. Red pitaya, white pitaya and papaya fruits were obtained from a local plantation in Lembah Bidong Setiu, Terengganu, Malaysia. The fruits were carefully washed under running tap water, dried with a soft cloth and the skin peeled; the fresh flesh was then cut into small pieces (1.5 cm x 1.5 cm x 1.5 cm). Lipids were removed from the sample (500 g) using petroleum ether (boiling

range, 37-55°C). The defatted fruit samples were stored at a temperature of 4°C for later use.

The optimal conditions selected for the extraction of oligosaccharides in both flesh and peel of red pitaya, white pitaya and papaya was carried out as follows: a total of 1.0 g of fruit samples were extracted 3 times with 10 mL/50% ethanol-water at a ratio of 10:1 (solvent to fruit extracts) in a water bath at a temperature of 50°C for 60 minutes. After each extraction, the samples were centrifuged at 2500g for 20 minutes. Supernatants from three cycles of extraction were combined and concentrated by using a rotary vacuum evaporator (Heidolph, Germany), and then dissolved with 5.0 mL of the mobile phase of HPLC (acetonitrile-water 75:25, v/v, HPLC grade of acetonitrile). Before injection, all samples were filtered through a 0.45 µm millipore membrane.

HPLC Analysis of Oligosaccharides

The separation and quantification of oligosaccharides from both flesh and peel of red pitaya, white pitaya and papaya were carried out by the Shimadzu HPLC system (Shimadzu, Japan), which consists of a pump and a 10A refraction index detector. Both sugar compound extraction and different standards of sugars were separated on a LiChroCART® 250-4 LiChrosper® NH2, 5µm column (Merck, Germany), using acetonitrile-water (75:25, v/v) as the mobile phase at a flow rate of 1.0 mL/min (temperature of 65°C). The injection volume sample was 20 µL.

Sugar and oligosaccharide compounds in both flesh and peel of red pitaya, white pitaya and papaya were identified by comparing the retention times of sugars (glucose, fructose and sucrose), standard oligosaccharides (raffinose, stachyose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose) and fructooligosaccharides (Sigma, Co. Chemical, St Louis, USA) and used as reference for quantification.

Identification and Quantification of Sugars and Oligosaccharides by High Performance Liquid Chromatography (HPLC)

For identification and quantification of sugars by HPLC, the peak of glucose, sucrose and fructose was identified using two techniques: comparing the retention times and the spiking test with the external HPLC standard for pure glucose, sucrose and fructose (Sigma, Co. Chemical, St Louis, USA). 10mg of glucose, sucrose and fructose was weighed and dissolved in pure water (HPLC grade) to give a stock solution of 100 µg/mL.

The solution was stored in a brown bottle and kept as stock in the fridge (at a temperature of 4-5°C). Peaks for oligosaccharide composition was identified on the chromatogram by comparing the retention time and spiking test with oligosaccharide standard kits which contain raffinose, stachyose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose (Sigma, Co. Chemical, St Louis, USA) dissolved in HPLC pure water (1 mg/mL).

HPLC grade of acetonitrile was added to each solution to obtain a composition similar to that of the mobile phase. The peak for fructooligosaccharide was identified by comparing the retention time and spiking test of FOS external standards (Sigma, Co. Chemical, St Louis, USA). 1mg of FOS was weighed and dissolved in pure HPLC water and acetonitrile to give a stock solution (100 µg/mL). The solution was stored in a brown bottle and kept as stock in the fridge (at a temperature of 4-5°C).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) in replicates of ten. Statistical analysis was performed with single factor and one way ANOVA to identify the significant differences in oligosaccharide composition in red pitaya, white pitaya and papaya using high performance liquid chromatography analysis (HPLC).

RESULTS AND DISCUSSION

Non-digestible oligosaccharides are low molecular weight carbohydrates which are intermediate in nature between simple

sugars and polysaccharides, and can be obtained by direct extraction from natural sources, or produced by chemical processes by hydrolyzing polysaccharides, or by enzymatic and chemical synthesis from disaccharides [9]. These compounds contain important physicochemical and have physiological properties beneficial to the health of consumers, thus, their use as food ingredients has rapidly increased.

Some of the beneficial properties are non-cariogenic, a low caloric value and the ability to stimulate the growth of beneficial bacteria in the colon. They are associated with a low risk of infection and diarrhea, and improves the immune system response. Moreover, due to the decrease in intestinal pH caused by the fermentation of non-digestible oligosaccharides, this provokes a reduction in pathogen flora, and increases bifidobacteria population and the availability of minerals [10].

The oligosaccharides can be classified according to their molecular size or degree of polymerization into monosaccharides, oligosaccharides or polysaccharides. The majority of non-digestible oligosaccharides available are in the form of food ingredients. This includes carbohydrates, in which the monosaccharide unit is fructose, galactose, glucose and/or xylose, and is known to promote the growth of beneficial bacteria in the colon, mainly the Bifidobacteria species and thus recognized as prebiotics [11].

Physical Properties of Flesh and Peel of Red Pitaya, White Pitaya and Papaya

The physical properties of the flesh of red pitaya, white pitaya and papaya are shown in the Table 6.1. The results show that the average weight of red pitaya flesh is 298.57 ± 1.80 g, white pitaya flesh (315.23 ± 6.70 g) and papaya (1050.36 ± 3.40 g). The average weight of red pitaya peel is 98.68 ± 6.90 g, white pitaya peel (100.90 ± 2.50 g) and papaya peel (313.85 ± 4.30 g). The results also showed the diameter of red pitaya as 13.35 ± 1.30 cm, white pitaya (12.65 ± 2.60 cm) and papaya (25.85 ± 1.10 cm). In general, the papaya fruit shows significantly higher measurements for weight and diameter as compared to red pitaya and white pitaya.

An ovoid-oblong berry pyriform or almost cylindrical, large, fleshy, juicy, grooved along the upper longer side, green yellow to yellow or yellow-orange colour when ripen, single cell of orange or reddish internal colour with many parietal seeds and a length of 10-25 cm or longer and 7-15 cm or more in diameter. Generally, the fruit is melon-like, oval to nearly round, somewhat pyriform, or elongated club-shaped, 15-50cm long and 10-20cm thick; and weighing up to 9 kg. Semi-wild (naturalized) plants bear miniature fruits 2.5-15 cm long. The skin is waxy and thin but fairly tough. When the fruit is green and hard it is rich in white latex. As it ripens, it becomes light or deep-yellow externally and the thick wall of succulent flesh becomes aromatic, yellow, orange or various shades of salmon or red. It is then juicy, sweetish and somewhat like a cantaloupe in flavor; in some types quite musky.

Attached lightly to the wall by soft, white, fibrous tissue, the seeds are usually numerous, small, black, ovoid, corrugated, and peppery about 3/16 in (5 mm) long, each coated with a transparent, gelatinous aril. The ones commonly found in the market usually average about 7 inches and weigh about one pound. Their flesh is a rich orange color with either yellow or pink hues. Inside the inner cavity of the fruit are black, round seeds encased in a gelatinous-like substance. Papaya seeds are edible, although their peppery flavor is somewhat bitter [12].

The white pitaya is ovoid in shape, with a red peel covered with short triangular bracts. The scales turn from green to red when ripe while the skin peels easily. Pulp colour varies from white to red or purplish, depending on the species and the variety. Numerous small edible seeds which resemble sesame seeds could be found embedded within the pulp. Fruit length is 15-22 cm, diameter is 8 - 11 cm and the fruit weighs 300g - 800g. Taste could range from slightly sour to sweet with 14% consisting of soluble solids during harvest, time.

Table 1: Physical properties of the flesh and peel of red pitaya, white pitaya and papaya

Characteristic	Red Pitaya	White Pitaya	Papaya
*Fruit weight (g)			
Flesh	298.57 ± 1.80 ^a	315.23 ± 6.70 ^b	1050.36 ± 3.40 ^c
Peel	98.68 ± 6.90 ^a	100.90 ± 2.50 ^a	313.85 ± 4.30 ^c
*Fruit characteristic (cm)			
Length	13.35 ± 1.30 ^a	12.65 ± 2.60 ^a	25.85 ± 1.10 ^b
Diameter	11.36 ± 1.20 ^a	10.67 ± 1.90 ^a	23.57 ± 2.30 ^b
*Total soluble solid			
Refractor (Brix)	16.34 ± 0.31 ^b	14.37 ± 0.67 ^a	9.56 ± 0.85 ^c
Flesh	7.43 ± 0.10 ^a	9.19 ± 0.12 ^c	8.35 ± 0.20 ^b
Peel			
Dry matter (%)	13.01 ± 0.82 ^b	12.83 ± 0.21 ^a	8.97 ± 0.15 ^c
Flesh	8.10 ± 0.48 ^a	5.19 ± 0.09 ^b	7.07 ± 0.10 ^c
Peel			
pH		4.59 ± 0.81 ^a	5.54 ± 0.46 ^c
Flesh	5.27 ± 0.97 ^b	6.59 ± 0.16 ^b	6.54 ± 0.13 ^b
Peel	6.91 ± 0.52 ^a		

Different letters in the same row mean a significant difference (p≤0.05).
* Average of 20 fruits
** Average of fifth replicate analyses by HPLC

The pitaya fruit plant grows well in tropical and subtropical climates. Shading of 30% is required if the temperature goes above 100°F. Extreme exposure to sunlight could lead to sun burn of the vines and too much shading would result in low production [12] and quality of fruits [13].

The red pitaya shape is the same as the white pitaya fruit, except the shape is rather ovoid with a diameter of 10 – 15 cm and weighing approximately 250–600 g in weight. The shape is ovoid and covered with scales that vary in size. The texture of the flesh is pleasant and has many edible black seeds [14].

The measurement of physical properties in the flesh and peel of red pitaya, white pitaya and papaya are also presented in Table 1. The result showed that the total soluble solid as measured by brix concentration in both flesh and peel of red pitaya is 16.34 ± 0.31 brix and 9.19 ± 0.12 brix and this is significantly higher compared to both flesh and peel of white pitaya (14.37 ± 0.67 brix; 7.43 ± 0.10 brix) and papaya (9.56 ± 0.85 brix; 8.35 ± 0.20 brix).

Both flesh and peel of red pitaya (13.01 ± 0.82%; 8.10 ± 0.48%) are significantly higher in dry matter as compared with white pitaya (12.83 ± 0.21%; 5.19 ± 0.09%) and papaya (8.97 ± 0.15%; 7.07 ± 0.10%). The pH value is also significantly different for both flesh and peel of red pitaya (5.27 ± 0.97; 6.91 ± 0.52) with white pitaya (4.59 ± 0.81; 6.59 ± 0.16) and papaya (5.54 ± 0.46; 6.54 ± 0.13).

The results from this study have a similar pattern with the study done by Ruzainah *et al.* [14] and Mohd Adzim Khalili *et al.* [15]. The study reported that the percentage of dry matter in red pitaya is significantly higher than white pitaya. According to

Jamilah *et al.*, [16], red pitaya peel was reported to have range of pH values 5.06 – 6.87, total soluble solid (6.00 brix) and proportion weight almost 35% from the fruit weight. Wichienchot *et al.*, [17] reported the mean value of red pitaya length 127.00 ± 0.55 and 134.00 ± 5.00, the fruit diameter of red pitaya (66.00 ± 4.0) was significantly lower than white pitaya (94.00 ± 9.00). The flesh weight of red pitaya (215.00 ± 35.00 g) was significantly lower than flesh of white pitaya (305 ± 0.75). The weight of red pitaya peel is 75.00 ± 25.00 g significant lower than white pitaya peel (100.00 ± 30.00 g).

The degree of sweetness of red pitaya flesh as measured by brix (14.80 ± 0.75 Brix) is much higher than the white pitaya flesh (12.50 ± 0.97 Brix). The previous study done by Ming and Chin[19], reported that the measurement of soluble solids in various parts of the pitaya fruits in Taiwan, showed the white pitaya had a higher soluble solid content than red pitaya. This might be due to the different variety or agricultural practices that has been planted in Taiwan.

Composition of Sugars in Both Flesh and Peel of Red Pitaya, White Pitaya and Papaya.

The composition of sugars in the flesh and peel of red pitaya, white pitaya and papaya was determined and identified by comparing peak sugar areas of the samples to that of the standard solutions (Sigma, Co. Chemical, St Louis, USA). From the HPLC analysis, the retention time for glucose, sucrose and fructose was RT 9.156 min, RT 10.388 min and RT 7.375 min respectively for both sample and standards. The quantification of glucose, sucrose and fructose used the formulation proposed by Tee and Lim [18].

Table 2: Sugar composition in both flesh and peel of white pitaya, red pitaya and papaya (mg/100 g e.p.)

Characteristic	Red pitaya	White pitaya	Papaya
Flesh			
Glucose	19.96 ± 0.10 ^c	12.67 ± 0.30 ^b	10.02 ± 0.09 ^a
Fructose	15.09 ± 0.05 ^c	10.89 ± 0.07 ^b	5.05 ± 0.02 ^a
Sucrose	13.97 ± 0.01 ^c	12.62 ± 0.10 ^b	3.01 ± 0.06 ^a
Peel			
Glucose	3.60 ± 0.02 ^c	2.07 ± 0.13 ^b	1.02 ± 0.05 ^a
Fructose	2.09 ± 0.07 ^c	1.89 ± 0.01 ^b	1.05 ± 0.01 ^a
Sucrose	1.97 ± 0.03 ^b	1.62 ± 0.10 ^b	1.01 ± 0.06 ^a

Different letters in the same row means a significant difference (p≤0.05).
* Average of 20 fruits
** Average of fifth replicate analyses by HPLC

The composition of glucose, sucrose and fructose in both flesh and peel of red pitaya, white pitaya and papaya is shown in Table 2. Glucose, sucrose and fructose concentrations in red pitaya flesh (19.96 ± 0.10 mg; 13.97 ± 0.01 mg; 15.09 ± 0.05 mg) was significantly ($p < 0.05$) higher than white pitaya flesh (12.67 ± 0.30 mg; 12.62 ± 0.10 mg; 10.89 ± 0.07 mg) and papaya flesh (10.02 ± 0.09 mg; 03.01 ± 0.06 mg; 05.05 ± 0.02 mg). Whereas, the composition of glucose, sucrose and fructose concentrations in red pitaya peel (3.60 ± 0.02 mg; 1.97 ± 0.03 mg; 2.09 ± 0.07 mg) was significantly ($p < 0.05$) higher than white pitaya peel (2.07 ± 0.13 mg; 1.62 ± 0.10 mg; 1.89 ± 0.01 mg) and papaya peel (1.02 ± 0.05 mg; 1.01 ± 0.06 mg; 01.05 ± 0.01 mg). The results of this study contradicts the previous study done by Wichienchot *et al.*, [17]. He has reported the quantitative determination of sugars in red pitaya flesh and white pitaya flesh. Glucose, fructose and sucrose are the major soluble sugars in the flesh of the pitaya fruit, while the content of sucrose accounted for only 3.80 – 9.60% of the total sugars. As a comparison, the pulp of prickly pear fruit had glucose and fructose present in almost equal amounts [20]. Takahata *et al.* [21] suggested that invertase is a contributing factor in the conversion of sucrose to same amounts of accumulated glucose and fructose. It was a different case with the red pitaya fruit, as the glucose and fructose present were in different amounts, suggesting that amylase may play an important role in sugar metabolism in the pitaya fruit.

Composition of Sugars in Both Flesh and Peel of Red Pitaya, White Pitaya and Papaya.

The oligosaccharide composition in both flesh and peel of red pitaya, white pitaya and papaya (Table 3) was determined and quantified

Table 3: Oligosaccharide composition in both flesh and peel of white pitaya, red pitaya and papaya (ug/100 g e.p.)

Characteristic	Red pitaya	White pitaya	Papaya
Flesh			
Raffinose	324.57 ± 3.80^c	204.23 ± 2.70^b	154.36 ± 3.40^a
Stachyose	283.58 ± 4.30^b	249.43 ± 2.50^a	252.68 ± 6.90^a
Maltotriose	304.23 ± 2.90^c	154.02 ± 1.60^a	254.81 ± 4.30^b
Maltotetraose	102.79 ± 2.60^b	89.32 ± 6.10^a	76.52 ± 8.90^a
Maltopentaose	344.52 ± 6.80^c	204.79 ± 5.90^b	107.21 ± 7.10^a
Maltohexaose	101.02 ± 2.30^c	54.69 ± 3.40^a	70.45 ± 1.90^b
Maltoheptaose	45.89 ± 1.90^b	20.67 ± 0.60^a	15.67 ± 1.10^a
Fructo-oligosaccharides	149.32 ± 5.90^c	104.92 ± 3.20^a	124.38 ± 3.20^b
Peel			
Raffinose	32.59 ± 0.28^b	24.23 ± 0.97^a	24.36 ± 0.64^a
Stachyose	30.23 ± 0.38^b	29.43 ± 2.5^b	23.12 ± 2.13^a
Maltotriose	28.51 ± 1.30^c	14.52 ± 1.62^a	24.98 ± 2.09^b
Maltotetraose	12.79 ± 0.26^b	08.29 ± 0.16^a	7.25 ± 0.96^a
Maltopentaose	104.45 ± 6.89^c	94.79 ± 5.90^b	87.34 ± 7.19^a
Maltohexaose	10.98 ± 0.35^c	05.01 ± 0.14^a	07.45 ± 1.01^b
Maltoheptaose	15.09 ± 1.09^c	10.67 ± 0.56^a	12.67 ± 1.91^b
Fructo-oligosaccharides	29.22 ± 0.89^c	14.92 ± 0.52^a	20.18 ± 0.23^b

Different letters in the same row mean a significant difference ($p \leq 0.05$).
 * Average of 20 fruits
 ** Average of fifth replicate analyses by HPLC

From the HPLC analysis, the retention time for maltotriose, maltotetraose and maltopentaose are RT 6.584 min, RT 18.354 and RT 20.738 min respectively for both sample and standards. The quantification of maltotriose, maltotetraose and maltopentaose used the formulation proposed by Tee and Lim [18]. From this study, it shows that red pitaya flesh has a significantly higher composition of maltotriose, maltotetraose and maltopentaose (304.23 ± 2.90 ug; 102.79 ± 2.60 ug; 344.52 ± 6.80 ug) content as compared to white pitaya flesh (154.02 ± 1.60 ug; 89.32 ± 6.10 ug; 204.79 ± 5.90 ug) and papaya flesh (304.23 ± 2.90 ug; 76.52 ± 8.90 ug; 107.21 ± 7.10 ug). Whereas the composition of maltotriose, maltotetraose and maltopentaose content in red pitaya peel (28.51 ± 1.30 ug; 12.79 ± 0.26 ug; 104.45 ± 6.89 ug) was found to be significantly higher than white pitaya peel (14.52 ± 1.62 ug; 08.29 ± 0.16 ug; 94.79 ± 5.90 ug) and papaya peel (24.98 ± 2.09 ug; 7.25 ± 0.96 ug; 87.34 ± 7.19 ug).

by comparing peak areas of sugar samples to those of the standard solutions (Sigma Aldrich, USA). From the HPLC analysis, retention time for raffinose and stachyose were RT 4.935 min and RT 5.775 min for both the sample and the standards. The quantification of raffinose and stachyose used the formulation proposed by Tee and Lim [18]. The composition of raffinose and stachyose in red pitaya flesh (324.57 ± 3.80 ug; 283.58 ± 4.30 ug) was found to be significantly higher than white pitaya flesh (204.23 ± 2.70 ug; 249.43 ± 2.50 ug) and papaya flesh (154.36 ± 3.40 ug; 252.68 ± 6.90 ug). Whereas, the composition of raffinose and stachyose contents (32.59 ± 0.28 ug; 30.23 ± 0.38 ug) in red pitaya peel was significantly higher than white pitaya peel (24.23 ± 0.97 ug; 29.43 ± 2.50 ug) and papaya peel (24.36 ± 0.64 ug; 23.12 ± 2.13 ug).

According to Wong and Jenkins [22], the fruit's flesh is the best source of raffinose and stachyose as compared to fruit peel. Among the oligosaccharides, the most important are raffinose and stachyose as these naturally occurring oligosaccharides are present as constituents of glycoproteins and glycolipids. These two examples of oligosaccharides belong to alpha galactosyl derivatives of sucrose. It can be broken down by the β galactosidase enzyme. This enzyme is not found in the human digestive tract or in tracts of other monogastric animals like pigs and poultry. Hence, the raffinose group of oligosaccharides (RFO) remains undigested in the stomach and upper intestine. In other parts of the intestine, the RFOs with the help of gut flora (intestinal bacteria) are fermented producing carbon dioxide (CO₂), methane and/or hydrogen (H₂).

Therefore, comparatively the composition of maltotriose, maltotetraose and maltopentaose in the fruit's flesh is significantly higher in value than the peel of the fruit. The maltotriose, maltotetraose and maltopentaose is further split to component sugars and absorbed through the action of brush border sucrose-isomaltase while lactose is split by lactase. Glucose is absorbed by 'active transport', while fructose and galactose are by 'active transport' facilitated by diffusion. The red pitaya flesh seems to be an excellent source of maltotriose, maltotetraose and maltopentaose, which could be exploited commercially by extracting it in their natural state [17].

From the HPLC analysis, the retention time for maltohexaose, maltoheptaose and fructo-oligosaccharides are RT 24.027 min, RT 28.272 and RT 12.754 min respectively, for both sample and standards. The quantification of maltotriose, maltotetraose and maltopentaose used the formulation proposed by Tee and Lim²⁰. The composition of

maltohexaose, maltoheptaose and fructo-oligosaccharides in both of flesh and peel of red pitaya was significantly higher than in both flesh and peel of white pitaya and papaya.

Maltohexaose, maltoheptaose and fructo-oligosaccharides (FOS) composition for both flesh (101.02 ± 2.30 ug; 45.89 ± 1.90 ug; 149.32 ± 5.90 ug) and peel (10.98 ± 0.35 ug; 15.09 ± 1.09 ug; 29.22 ± 0.89 ug) of red pitaya was significantly higher than in flesh (54.69 ± 3.40 ug; 20.67 ± 0.60 ug; 104.92 ± 3.20 ug) and peel (5.01 ± 0.14 ug; 10.67 ± 0.56 ug; 14.92 ± 0.52 ug) of white pitaya, and flesh (70.45 ± 1.90 ug; 15.67 ± 1.10 ug; 124.38 ± 3.20 ug) and peel (7.45 ± 1.01 ug; 12.67 ± 1.91 ug; 20.18 ± 0.23 ug) of papaya.

The results of this study contradicts the results of previous studies done by Wichienchot *et al.*, [17] who reported that the composition of maltohexaose, maltoheptaose and fructo-oligosaccharides in the fruit's peel is higher than that in the fruit's flesh. The findings of this study are similar with findings by Wong and Jenkins [22], in that the fruit's flesh is the best source of maltohexaose, maltoheptaose and fructo-oligosaccharides compared to the fruit's peel.

Traditionally, the amount of carbohydrate available for colonic bacterial fermentation is determined by the amount of dietary fibre present in foods. However, some of the 'available carbohydrate' (i.e., "available" for small intestinal absorption: total carbohydrate minus dietary fibre) in many foods may escape digestion in the small intestine in appreciable amounts and become available for fermentation by the colonic micro flora [23, 24].

Early studies in ileostomates were conducted to determine carbohydrate losses with different types of foods that vary in fibre [25] and available carbohydrate content [26]. Certain foods have been related to a greater proportion of carbohydrate loss compared to others: specifically lentils and other legumes; the β -glucan-containing cereals such as oat bran and barley; and pumpernickel bread, where the whole grain structure is preserved [27].

Prebiotics, such as oligofructose and inulin, are emerging as functional foods associated with improvements for better health. Administration of these dietary components promotes the growth of specific bacteria, especially bifidobacteria and lactobacillus, which have defined metabolic functions [26]. Studies involving patients with ileostomies have shown that 88% and 89% of inulin and oligofructose, respectively, have been recovered in their effluents [27]. These oligosaccharides are examples of carbohydrates that are almost entirely not digested in the small intestine, a characteristic that has led to growing research on their effects on colonic and systemic health [28].

CONCLUSION

Previous study on antioxidant capacity dan radical scavenging activity were showed that extracts of the flesh and peel of red pitaya, white pitaya and papaya have powerful antioxidant activity against various antioxidant systems in vitro [29] and crude compound from both flesh and peel of red pitaya, white pitaya and papaya have great potential as antimicrobial compounds against microorganisms and can significantly inhibit the growth of *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Listeria monocytogenes* [30].

The results of the present study have revealed that both flesh and peel of red pitaya, white pitaya and papaya are a good source of prebiotic oligosaccharides. The composition of prebiotic oligosaccharide is significantly higher in the fruit's flesh as compare to the fruit's peel. The flesh of red pitaya was found to have reasonably high proportions of prebiotic oligosaccharides as compared to white pitaya and papaya.

This study proved that, the red pitaya fruit represents a rich source of prebiotic oligosaccharides. It should be regarded as a valuable new source of prebiotic oligosaccharide with the potential for use as an economical value-added ingredient to be used as a substrate together with probiotics for the development of functional foods to assist in the prevention of chronic diseases.

Moreover, the red pitaya, white pitaya and papaya can be used as an easily accessible source of natural antioxidant and as a possible food supplement or in pharmaceuticals application. Further studies are

necessary to identify the primary active prebiotic oligosaccharide compounds in this commercially promising subtropical fruit.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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