International Food Research Journal 23(6): 2581-2587 (December 2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Digestibility, fecal fermentation and anti-cancer of dragon fruit oligosaccharides

[1]Dasaesamoh, R., [1,2]Youravong, W. and [3,4*]Wichienchot, S.

[1]Department of Food Technology, Faculty of Agro-Industry, [2]Membrane Science and Technology Research Center, [3]Interdisciplinary Graduate School of Nutraceutical and Functional Food, [4]The Excellent Research Laboratory for Cancer Molecular Biology, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

Article history

Received: 8 September 2015
Received in revised form: 31 January 2016
Accepted: 21 February 2016

Keywords

Dragon fruit flesh
Oligosaccharides
Prebiotic
Caco-2 cells

Abstract

Dragon fruit is becoming more popular due to their nutritional benefits. It has been reported as a potential source of natural prebiotic since it contains oligosaccharides. This research aims to evaluate prebiotic properties of oligosaccharides from dragon fruit's flesh. The oligosaccharides are non-digestible oligosaccharides since they resisted hydrolysis by human α-amylase in mouth, artificial human gastric juice in stomach and human α-amylase with sucrase, giving maximum hydrolysis of 6.7%, 0.6% and 4.81%, respectively. Fecal fermentation of the oligosaccharides showed increase in the populations of bifidobacteria, lactobacillus and decrease in the populations of bacteroides and clostridium. Also, the fecal fermentation of the oligosaccharides had a positive prebiotic effect with prebiotic index (PI) of 0.41. Acetic acid, lactic acid, propionic and butyric acid were produced at concentrations of 860, 265, 15.95 and 29.63 mM, respectively. The mixture of these short-chain fatty acids has the propensity to inhibit Caco-2 cells which it has potential for risk reduction in colon cancer.

Introduction

Dragon fruit (pitaya) is the fruit of the cactus species, in the genus Hylocereus and Stenocereus. Generally, pitaya comes in three types; *Hylocereus polyrhizus* (red pulp with red-magenta skin, *Hylocereus megalanthus* (white pulp with yellow skin) *Hylocereus undatus* (white pulp with magenta skin) (Ariffin *et al.*, 2008). In Thailand, one of widely grown varieties is magenta skin with white-flesh (pulp). Other varieties that have been commercialized are *Hylocereus polyrhizus* (red-magenta skin with red-flesh) and *Hylocereus megalanthus* (yellow skin with white flesh) (Barbeau, 1990). Currently, there is much interest in developing this crop for export beyond the local sale in Asian markets of Singapore, Hong Kong, Taiwan, Philippines, Malaysia and Thailand (Hoa *et al.*, 2006).

The dragon fruit pulp consists of highly viscous carbohydrate fibers (cellulose, hemicellulose and simple sugars), vitamin C, minerals and polysaccharides such as starch and pectin (Nur'aliaa *et al.*, 2010a). Dragon fruit juices are turbid, very viscous and contain colloidal suspension. Therefore, enzymatic treatment with pectinase is an effective way to reduce the pectin in the fruit juices because pectinase has the ability to hydrolyze pectin (Sin *et al.*, 2006), thereby obtaining desired oligosaccharides. The chemical compositions of oligosaccharides in dragon fruit 's flesh are fructooligosaccharides with DP 3-5 (Wichienchot *et al.*, 2010). Khalili *et al.* (2014) reported that dragon fruit 's flesh oligosaccharides consisted of raffinose, stachyose, maltotriose, maltotetriose, maltopentaose, maltohexaose, maltoheptaose and fructooligosaccharides. Although, fractans-type oligosaccharides can be found in Jerusalem and artichoke but the chemical structure, linkages and degree of polymerization are different from dragon fruit flesh oligosaccharides. Thus, prebiotic property of dragon fruit flesh oligosaccharide may vary from other sources i.e. plants and enzymatic biosynthesis.

Prebiotics are non-digestible oligosaccharides that beneficially affect the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health (Gibson and Roberfroid, 1995). Various fruits and vegetables were reported as potential sources of natural prebiotics. Nowadays, chicory and artichoke roots have been extensively used as natural sources of inulin and oligofructose in the commercial production of prebiotics, giving oligosaccharide yields in the range of 18–20% (Gibson and Rastall, 2006). Extraction of oligosaccharides from dragon

*Corresponding author.
Email: *santad.w@psu.ac.th*

fruit's flesh using ethanol and water as solvents and study of its prebiotic properties has been reported previously (Wichienchot *et al*., 2009). However, evaluation of digestibility, prebiotic properties in a batch culture, and inhibition of colon cancer cells (Caco-2) have not been investigated.

Materials and Methods

Raw material, chemicals, media, enzyme and microorganism

White-flesh dragon fruit *(H. undatus)* was obtained from a local plantation in Hat Yai, Songkhla, Thailand. All chemical reagents and pectinase from Aspergillus niger were obtained from Sigma-Aldrich Co. Ltd. Cheesecloth bags tailored to 1×1 mm. size. *S. cerevisiae* (baker's yeast) strain TISTR 5019 was obtained from a National Microbial Collection, National Research Council of Thailand (NRCT), Bangkok, Thailand. Colonic cancer cell (Caco-2) was purchased from ATCC HTB-37, DNA probes were purchased from Sigma-Aldrich Co. Ltd.

Preparation of oligosaccharides from dragon fruit's flesh

White-flesh dragon fruits were used as raw material. The dragon fruit was peeled, and cut into a cube of 1 cm^3. The flesh was mixed with water at ratio of flesh to water 1: 2. Addition of pectinase from *Aspergillus niger* at concentration of 7 unit/ml of final reaction mixture. The reaction was carried out in 3 L stirred reactor tank at controlled temperature of 40±2°C (Nur' aliaa *et al*., 2011) using circulating water bath with continuous shaking (250 rpm) for 45 minutes. Then, dragon fruit's flesh sample was boiled for 5 minutes to inactivate the enzyme. The sample was filtered to remove the dark seeds by cheese cloth. Aseptically, the extract was dispensed into 250 ml of Erlenmeyer flask and inoculated with 18-hour grown 1×10^6 CFU/ml S. cerevisiae TISTR 5019 at concentrations of 2.5% (w/v). Fermentation was carried out for 72 hours on shaker (180 rpm), after which cultured samples were centrifuged (10,000×g) for 10 minutes to remove the yeast cells. Yeast fermentation was carried out to remove mono-/ di-saccharides in the oligosaccharides mixture thus higher purity of dragon fruit flesh oligosaccharides were obtained. The supernatants were analyzed for sugar content, oligosaccharides, monosaccharides and disaccharides by HPLC with RI detector. Chemical composition of

Digestibility of enzyme and acid in artificial digestive tract system

A sample of 30 g oligosaccharides (prepared accordance with Dasaesamoh, 2014) was suspended in 500 ml artificial saliva (NH_2PO_4, 0.636 g/l; NH_4NO_5, 0.328 g/l; NaCl, 1.594 g/l; KCl, 0.202 g/l; $K_5C_6H_5O7.H_2O$, 0.308 g/l; $C_5H_5N_4O_5Na$, 0.021 g/l; H_2NCONH_2, 0.198 g/l; $C_5H_5O_5Na$, 0.146 g/l; porcine gastric mucin 30 g/l, pH 6.8). Sample (dragon fruit flesh's oligosaccharides) was incubated stepwisely under simulated mouth conditions, while human salivary α-amylase solution was added (Sigma A1031, Buchs, Switzerland) at pH 6.9 for 15 minutes. The sample was taken at 0, 5, 10, 15, 20, 30 and 40 minutes. Then, the sample was boiled for 5 minutes to inactivate the enzyme. Under simulated stomach conditions, the sample was adjusted to pH 2 with HCl. The sample was taken at 0, 10, 20, 30, 60, 90, 120, 180 and 240 minutes. Then, the sample was neutralized pH with 1 M NaOH. Under simulated small intestine conditions, the sample was added to obtain the final concentration of 0.75 unit/ ml porcine pancreatic (Type VI-B, Sigma) and 8,000 unit/ ml sucrase at pH 6.9 for 3 hours. The sample was taken at 0, 1, 2, 3, 4 and 5 hours. Degradation products were removed by sedimentation with 95% ethanol for 24 hours at 4°C, prepared and used for determination of prebiotic property in batch culture.

Stirred pH-controlled batch culture system

Stirred pH-controlled batch culture system consisted of glass vessel, pH-controller and facilities to support anaerobic fermentation. A sterile batch culture fermenter (300 ml capacity) was filled with sterile basal medium and pre-reduced overnight by purging with oxygen-free nitrogen gas. 100 ml fecal slurries were added to each fermenter and the closed fermenter maintained under a head space of oxygen-free nitrogen gas. Fermentation was carried out at 37ºC, magnetically stirred and culture pH was controlled at 6.8±0.1 by addition of 0.5 N NaOH or HCl through 24 hours. 5 ml of samples (fecal cultures) were taken at 0, 6, 12, 24 and 48 hours for enumeration of bacteria using FISH technique and short chain fatty acids (SCFA) were analyzed by HPLC. The prebiotic index (PI) of each sample (fecal cultures) tested at each time point was calculated following an equation given in analytical methods.

Fluorescence in situ hybridization (FISH) technique

A volume of 375 µl sample (fecal culture) was removed from the culture and added to 1.125 ml volume of filtered 4% (w/v) paraformaldehyde solution at pH 7.2, after which they were mixed and

stored at 4°C overnight to fix the cells. The fixed cells were washed twice in filtered PBS (pH 7) and resuspended in 150 µl filtered PBS. Ethanol (150 µl) was added and the sample was mixed and stored at 20°C for at least 1 hour or until use, but no longer than 3 months. The fixed cells were diluted to suitable concentration and 20 µl of the diluted mixture was transferred to a TEFLON/Poly-L-Lysine coated slide and placed on slide dryer at 45°C for 10-12 minutes until dried. The slide was soaked in a series concentration of ethanol 50%, 80% and 96% (v/v), respectively for 3 minutes each and placed on slide dryer. Pre-warm hybridization buffer was warmed at suitable hybridized temperature of each probe, after which it was filtered. The suitable volume of the mixture (i.e. 45 µl) was mixed with 5 µl of labeled probe DNA solution (50 ng /µl). Hybridization buffer (50 ml) was filled with probe mixture and slide was placed in hybridization oven at appropriate temperature for 4 h. The hybridized samples were washed in 50 ml wash buffer with appropriate hybridization temperature. Fluorescence microscope was fitted with appropriate filters for the DNA probe staining, minimum of 15 fields, each containing 10-100 cells, was counted for each slide.

Analysis of short-chain fatty acids

Short chain fatty acids (SCFA) including acetic, propionic, butyric acid and lactic acid were analyzed by HPLC. Samples (taken from stirred pH-controlled batch culture system) were centrifuged at 13,000 xg for 15 minutes, then supernatant was filtered through nylon filter (2.0 µm). The SCFA was determined by HPLC with BIO-RAD Aminex HPC-87 H Ion Exclusion column 300 mm×7.8 mm i.d. Mobile phase was 0.0005 M H_2SO_4, flow rate was 0.6 ml/min, oven temperature was 50°C and UV detector 215 nm. The concentration of SCFA was calculated by comparing the peak areas to respective standard curves of each SCFA using Chemstation program (Agilent Chemstation). The concentration of SCFA was calculated by comparing the peak areas to respective standard curves of each SCFA using lactic acid (2.5, 25 and 50 mM) acetic acid, propionic acid and butyric acid (1, 10 and 20 mM) as standards (Olano-Martin *et al*., 2000).

Anti-colonic cancer property of dragon fruit flesh oligosaccharides

The fermented broth in fecal batch culture was evaluated on its anti-cancer property. Samples (fecal cultures) were harvested at each time point and it were centrifuged at 13000 x g for 15 minutes to obtain cell-free broth. Cell-free broth sample was evaluated on the effectiveness of inhibiting carcinogenic cell. Human carcinogenic cell (1 ml) of Caco-2 was obtained from ATCC HTB-37 in liquid form. The cell was cultured in a plastic culture bottle supplemented with 6-8 ml of Eagle' Minimum Essential Medium (fecal bovine serum added). The mixture was incubated in CO_2 incubator at 37°C until the multiplication of cells reached $8 \times 10^4 - 1 \times 10^5$ cell/cm^2. Cell cultivation was performed in 96 – well plate with 2.0 ml of Eagle' Minimum Essential Medium. Carcinogenic cells (5×10^4 cell/ml) were added in the concavity plate and samples (0.1 ml) were filled into the plate at various concentrations. The mixture was incubated in CO_2 incubator at 37°C with 5% CO_2 atmosphere and 95% humidity for 24 h. The media was removed, after which 0.25% trypsin, 0.53 mM EDTA and MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide] (yellow substance) were added. Change in color was observed in the mixture; color changed to blue for living cells. Cell growth was measured with absorbance detection at 570 nm and converted to cell concentration by mean of calibration curve. The samples were measured in triplicate and the percentage of survival of carcinogenic cells were reported (Freshney, 2005).

Statistical analysis

Differences between bacterial counts at 0, 6, and 24 h of batch culture fermentations, were tested on their significance using paired t-tests, assuming equal variance and considering both sides of the distribution (two-tailed distribution). Differences were considered at 99% and 95% significance if $p< 0.01$ and $p< 0.05$, respectively using SPSS 16.0 for Windows software (Chicago, SPSS Inc.).

Results and Discussion

Purification of dragon fruit flesh oligosaccharides by yeast fermentation

Purification is a necessary step for the production of oligosaccharides from its mixture. In principle, purification methods of mixture of high molecular weight polysaccharides, oligosaccharides and sugars has 3 methods; physical, chemical and biological separation. In this study, biological separation by yeast fermentation had been applied to investigate removal of sugars from oligosaccharides in dragon fruit flesh. The results showed that sugars (glucose, fructose and sucrose) in the extract of dragon fruit flesh were completely removed at day 4 of yeast (*Saccharomyces cerevisiae* TISTR 5019) fermentation (Dasaesamoh, 2014). Moreover, the yeast fermentation did not affect the oligosaccharides contents in the extract (Figure

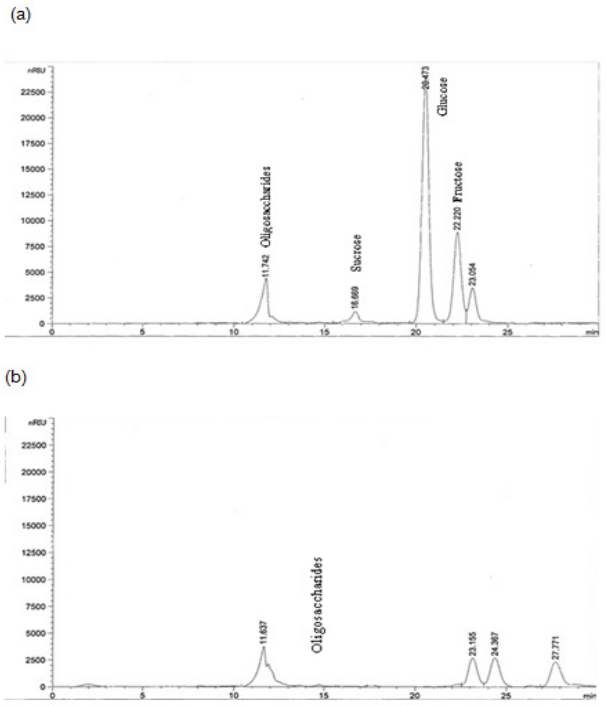

Figure 1. Chromatogram of the extract of dragon fruit flesh before (a) and after (b) purification by *S. cerevisiae* TISTR 5019 fermentation with the addition of urea, 0.1% (w/v)

1). The glucose, fructose, and sucrose in the extract were metabolized by yeast, then converted to ethanol and carbon dioxide according to report of Crittenden (2002). Therefore, purification of the extract by yeast fermentation to remove sugars was possible with no effect on the desired oligosaccharides. The yeast fermentation under these optimal conditions could completely remove glucose, fructose and sucrose without any effect on the oligosaccharides content. In comparison with others purification methods such as membrane technology, Goulas *et al.* (2002) have studied purification of commercial galacto-oligosaccharides with nanofiltration, and it was found that purity of galacto-oligosaccharides was 67-98%. Also when compared with commercial oligosaccharides including fructo-oligosaccharides (Raftilose® P95, Orafti, Belgium), transgalactosylated oligosaccharides (Oligomate® 55, Yakult, Japan), galacto-oligosaccharides and xylo-oligosaccharides, it was found out that their purity were 95%, 55%, 85% and 70% respectively (Georage *et al*., 1999). The purity of dragon fruit flesh oligosaccharides by yeast purification in this study was 99.9% (Dasaesamoh, 2014). Therefore, purification of oligosaccharides with yeast fermentation have higher purity than others purification methods.

Hydrolysis of dragon fruit flesh oligosaccharides in simulated mouth conditions

Oligosaccharides obtained from purification

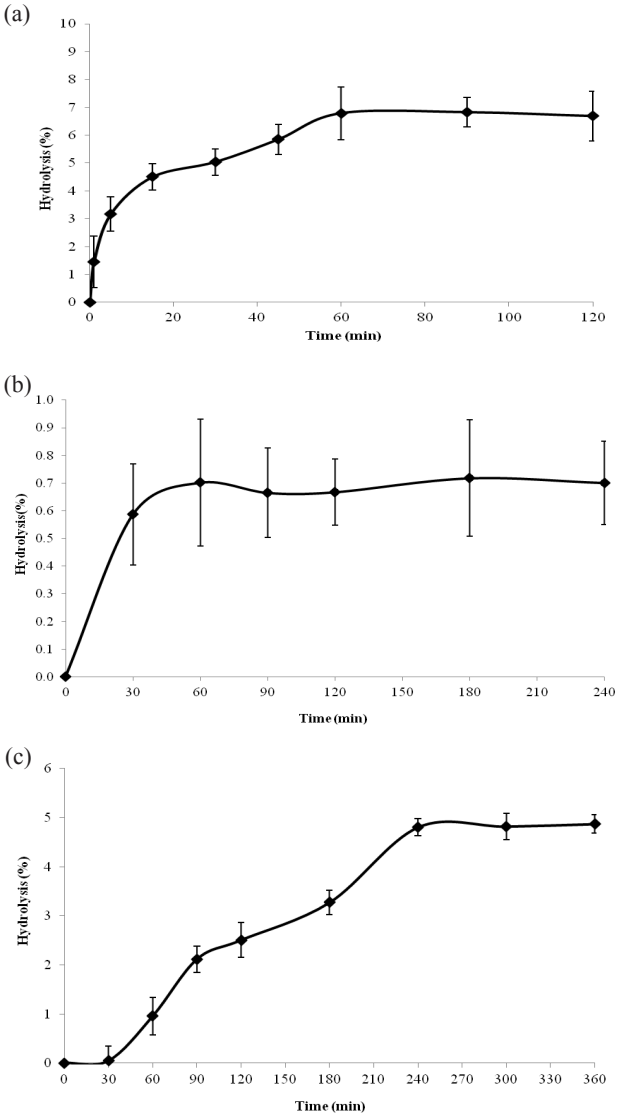

Figure 2. Percentage hydrolysis of dragon fruit flesh oligosaccharides by human salivary α-amylase (a), artificial gastric juice (b) and human pancreatic α-amylase and sucrase (c) incubated at 37°C for 2, 4 and 6 hours, respectively

with yeast fermentation were digested with artificial saliva juice containing human salivary α-amylase under simulated conditions in human mouth. It was found that percentage of hydrolysis increased with increasing incubation time. The maximum hydrolysis of oligosaccharide from dragon fruit's flesh in artificial saliva juice was 6.7% at 60 minutes (Figure 2a). It is estimated that 93% of dragon fruit flesh consumed will reach the stomach. Wichienchot *et al.* (2010) reported that oligosaccharides from dragon fruit's flesh (DP=3-4) was hydrolyzed in the mouth and small intestine for 10% and 30%, respectively. This is due to the presence of several enzymes in the small intestine such as maltase, lactase, sucrase, α-amylase and others which can hydrolyze α-D-(1,4) glycosidic linkage bond in the dragon fruit oligosaccharides.

Hydrolysis of dragon fruit flesh oligosaccharides in simulated stomach conditions

Under simulated conditions in human stomach, the hydrolysis of oligosaccharides occurred by random degradation of linkage between sugar moieties. Results showed a rapid increase in the percentage of hydrolysis when incubation time increased and unchanged thereafter 30 minutes. The maximum hydrolysis of oligosaccharides from dragon fruit's flesh in artificial human stomach was 0.6% at 30 minutes (Figure 2b). Thus 92.4% of the oligosaccharides is estimated to reach the small intestine.

Hydrolysis of dragon fruit flesh oligosaccharides in simulated small intestinal conditions

In simulated conditions mimicking small intestinal conditions, the hydrolysis of oligosaccharides by human α-amylase and sucrase were evaluated. The percentage of hydrolysis increased slowly when incubation increased and maximum hydrolysis of oligosaccharides from dragon fruit's flesh was 4.81% at 240 minutes (Figure 2e). Thus, 12.41% of the oligosaccharides was hydrolyzed throughout upper gastrointestinal tract and 87.59% of them are estimated to reach the colon.

Prebiotic property of oligosaccharides from dragon fruit's flesh in fecal batch culture fermentation

Oligosaccharides extracted from dragon fruit's flesh were used as carbon source for fecal fermentation in batch culture. It was found that at 0 hour, the numbers of bifidobacteria, lactobacillus, bacteroides, clostridium and eubacteria were 7.4, 7.6, 7.7, 8.2 and 8.4 Log cell/ml, respectively. After the oligosaccharides were added in batch culture and fermentation was performed for 24 hours, oligosaccharides increased the numbers of bifidobacteria and lactobacillus significantly ($p< 0.05$) to 8.30 and 8.25 Log cell/ml, respectively. In addition, numbers of bacteroides and clostridium decreased significantly ($p< 0.05$) to 7.03 and 7.15 Log cell/ml, respectively. Meanwhile, there was no significant ($p > 0.05$) in the numbers of eubacterium as shown in Figure 3. The prebiotic index (PI) represents a comparative relationship between the growth of "beneficial" bacteria, such as bifidobacteria, lactobacilli, eubacterium, and that of the "less desirable" ones, such as clostridia and bacteroides, in relation to the change in the total number of bacteria. For this study, the PI values obtained at 24 hour of incubation in oligosaccharides from dragon fruit's flesh was 0.41 lower than commercial prebiotic fructooligosaccharides (4.08)

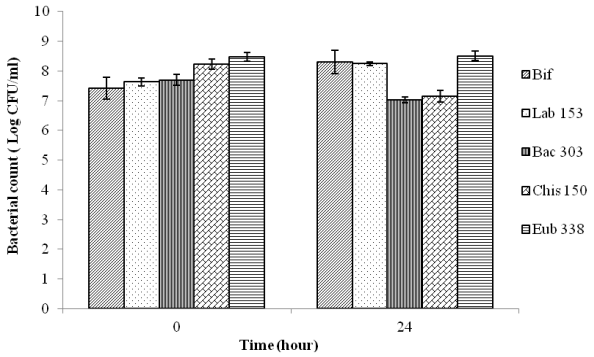

Figure 3. Bacterial populations change by fecal slurry fermentation of dragon fruit flesh oligosaccharides in stirred pH-controlled batch culture

at 24 hours of incubation (Mandalari *et al*., 2008). Prebiotic index of FOS from other sources showed that Jerusalem artichoke had PI value of 2.33 (Prakobpran, 2014), orange juice manufacturing by-product had PI value of 7.84 (Manderson *et al.*, 2005) and almond (*Amygdalus communis* L.) seeds had PI value as 4.43 (Mandalari *et al*., 2008). Prebiotic index of fructooligosaccharides from various sources may vary due to they have difference in the chemical compositions, type of linkages, molecular weight distributions and purity of oligosaccharides. In this study showed lower PI compared to commercial fructooligosaccharides from chicory. It may be due to the commercial fructooligosaccharides has higher content of non-digestible but fermentable oligosaccharides and also inulin.

Dragon fruit flesh oligosaccharides were metabolized by intestinal microbiota and subsequently converted into short chain fatty acids (acetic acid, propionic, butyric acid and lactic acid). The short chain fatty acids were analyzed by HPLC. It was found that acetic acid, lactic acid, propionic acid and butyric acid increased when incubation time increased (Table 1).

The ratio of acetate, propionate and butyrate in mammals vary from approximately 60:25:10 while the molar ratios of acetate: butyrate were 7.5:10 to 40:20 (Bergman, 1990). However, this depends on the type of oligosaccharides, source of fecal microbiota and fermentation time (Basson *et al.*, 1998). Increasing of rate of either acetate, propionate and butyrate, which may have a transmural trophic effect and preserve mucosal surface area from dysfunctional and atrophic colon in rats (Kissmeyer *et al*., 1995). An estimated, 60 – 70% of the energy of the epithelium of the colon is derived from SCFAs, particularly from butyrate (Scheppach *et al*., 1992). Butyrate is also responsible for some other important functions in the intestinal epithelium, such as prevention of certain types of

Table 1. Concentrations of short chain fatty acids derived by dragon fruit flesh oligosaccharides fermentation in stirred pH-controlled batch culture

Time	Short chain fatty acid (mM)			
	Acetic acid	Lactic acid	Propionic acid	Butyric acid
0	336.04 ± 1.39^{d}	114.92 ± 2.25^{c}	1.18 ± 0.04^{d}	0 ± 0.00^{c}
6	433.44 ± 23.75^{c}	183.77 ± 0.96^{b}	5.23 ± 0.44^{c}	13.30 ± 0.05^{b}
12	651.44 ± 5.01^{b}	267.38 ± 5.20^{a}	12.42 ± 0.83^{b}	27.10 ± 0.37^{a}
24	860.06 ± 29.64^{a}	265.71 ± 9.92^{a}	15.96 ± 0.31^{a}	29.63 ± 0.88^{a}

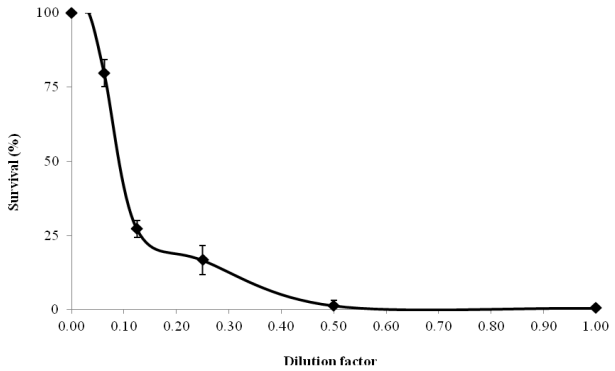

Figure 4. Percentage on survival of carcinogenic cell (Caco-2) in cell-free broth after fecal slurry fermentation of dragon fruit flesh oligosaccharides at various concentrations

colitis (Scheppach, 1994). Butyrate increases the activity of the heat labile alkaline phosphatase both in human follicular thyroid carcinoma cell (Schreck *et al.*, 1994) and endometrial cell lines (Fleming *et al.*, 1995). Furthermore, it represses thymidine kinase. Acetate increases colonic blood flow and enhances ileum motility (Scheppach, 1994). Propionic acid and butyric acid can promote health in humans according to previous study done by Macky and Gibson (1997). Metabolic end products such as short chain fatty acids excreted by probiotic may lower the gut pH to levels below those at which pathogens are able to effectively compete.

Anti-colonic cancer property of dragon fruit flesh oligosaccharides

The cell-free broth after batch fermentation of the oligosaccharides by fecal slurry for 24 hours was used to evaluate the effectiveness for inhibiting carcinogenic cell (Caco-2). It was found that percentage of survivor of Caco-2 decreased by increasing concentration of cell-free broth sample. The major components in cell-free broth sample were acetate, citrate, propionate and butyrate (Figure 4). This finding is in accordance with a previous report done by Scheppech and colleagues (1995). It has been reported that 1-5 mM butyric acid can inhibit the growth of Caco-2 cells and no inhibiting effect to normal intestinal cells at 10-60 mM. The mechanism on inhibiting tumor cell growth by butyric acid has been known. The process involves interruption of DNA methylation which in turn, affects the growth and proliferation of cancer cells (Archer *et al.*, 1998).

Conclusions

We have shown that oligosaccharides extracted from dragon fruit's flesh are candidate prebiotics since they partially resist upper gut digestion and also, these oligosaccharides have prebiotic property in batch culture fermentation by fecal microbiota. Furthermore, dragon fruit flesh oligosaccharides are also potential source of prebiotic for tropical or arid region. The oligosaccharides showed resistance to simulated conditions in the upper gut, whist promoting growth of bifidobacteria. This is the first report to show that cell-free broth of dragon fruit oligosaccharide fermentation by fecal microbiota has anti-colonic cancer property.

Acknowledgments

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission. Thanks to Faculty of Agro-Industry, Prince of Songkla University, for the supporting facilities.

References

Archer, S., Meng, S., Wu, J., Johnson, J., Tang, R. and Hodin, R. 1998. Butyrate inhibits colon carcinoma cell growth through two distinct pathways. Surgery

124: 248-253.

Ariffin, A. A., Bakar, J., Tan, C. P., Rahman, R. A., Karim, R. and Loi, C. C. 2009. Essential fatty acids of pitaya (dragon fruit) seed oil. Food Chemistry 14(2): 561–564.

Barbeau, G. C. 1990. La pitahaya rouge, un nouveau fruit exotique the red pitahaya, a new exotic fruit. Fruits 45: 141-147.

Basson, M. D., Emenaker, N. J. and Hong, F. 1998. Differential modulation of human (Caco-2) colon cancer cell line phenotype by short chain fatty acids. Proceeding of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine Royal Society of Medicine, New York.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiology Review 70: 567-590.

Crittenden, R. and Playne, M. 2002. Purification of food-grade oligosaccharides using immobilized cells of Zymomonas mobilis. Applied Microbiology Biotechnology 3: 297-302.

Dasaesamoh, R. 2014.Extraction and purification of oligosaccharide from dragon fruit's flesh and evaluation of prebiotic properties. Hat Yai, Thailand: Prince of Songkla University, MSc. thesis.

Fleming, H., Begley, M., Campi, T., Condon, R., Dobyns, K., Mcdonagh, J. and Wallace, S. J. 1995. Induction of alkaline phosphatase by butyrate in differentiating human endometrial cells. Cell Biochemistry 58: 509-516.

Freshney, R. I. 2005. Cultivation of animal cells: A Manual of Basic. 4th ed. John Wiley & Sons, Inc. 642.

Gibson, G. R. and Roberfroid, M. B. 1995. Dietary modulation of the human colonic micro biota: introducing the concept of prebiotics. Journal of Nutrition 125: 1401-1412.

Gibson, G. R. and Rastall, R. A. 2006. Prebiotic Development & Application. John Wiley & Sons, Ltd. 213-236.

Goulas, A. K., Kapasakalidis, P. G., Sinclair, H. R. and Rastall, R. A. 2002. Purification of oligosaccharides by nanofiltration. Journal of Membrane Science 209: 321-332.

Hoa, T. T., Clark, C. J., Waddell, B. C. and Woolf, A. B. 2006. Postharvest quality of dragon fruit *(Hylocereus undatus)* following disinfesting hot air treatments. Postharvest Biology and Technology 41: 62-69.

Khalili, R. M. A., Abdullah, A. B. C. and Manaf, A. A. 2014. Isolation and characterization of oligosaccharides composition in organically grown red pitaya, white pitaya and papaya. International Journal of Pharmacy and Pharmaceutical Sciences. 6(2): 131-136.

Kissmeyer-nielsen, P., Mortensen, F. V., Laurberg, S. and Hessov, I. 1995. Transmural trophic effect of short chain fatty acid infusions on atrophic, defunctioned rat colon. Diseases of the Colon and Rectum 38: 946-951.

Mandalari, G., Nueno-Palop, C., Bisignano, G., Wickham, M. S. J. and Narbad, A. 2008. Potential Prebiotic Properties of Almond (*Amygdalus communis* L.) seeds Applied Environmental Microbiology 74(14): 4264–4270.

Manderson, K., Pinart, M., Tuohy, K. M., Grace, W. E., Hotchkiss, A. T., Widmer, W., Yadhav, M. P., Gibson, G. R. and Rastall, R. A. 2005. *In vitro* determination of prebiotic properties of oligosaccharides derived from an orange juice manufacturing by-product stream. Applied Environmental Microbiology 71(20): 8383-8389.

Nur' aliaa, A. R., Siti, M. M. K., Tailip, F. S. and Liew, A. A. G. 2011. Effects of commercial pectinase application on selected properties of red pitaya juice. Journal of Food Process Engineering 5: 1523-1534.

Olano-Martin, E., Mountzouris, K. C., Gibson, G. R. and Rastall, R. A. 2000. In vitro fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. British Journal of Nutrition 83: 247-255.

Prakobpran, P. 2014.Production of fructooligosaccharides by fructosyltransferase from Jerusalem artichoke. Hat Yai, Thailand: Prince of Songkla University, MSc. thesis.

Scheppach, W., Sommer, H., Kirchner, T., Paganelli, G. M., Bartram, P., Christl, S., Ritchter, F., Dusel, G. and Kasper, I. I. 1992. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 103: 51-56.

Scheppach, W. 1994. Effects of short chain fatty acids on gut morphology and function. Gut Supplement 1: 35-38.

Scheppach, W., Bartram, H. P. and Richter, F. 1995. Role of short-chain fatty acids in the prevention of colorectal cancer. European Journal of Cancer Care 31: 1077-1080.

Schreck, R., Schneiders, F., Schlutzler, C. and Kohle, J. 1994. Retinoids stimulate type I iodothyronine 5'-deiodinase activity in human follicular thyroid carcinoma cell lines. The Journal of Clinical Endocrinology Metabolism 79: 791-798.

Sin, H. N., Yusof, S., Hamid, N. S. A. and Rahman, R. A. 2006. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. Journal of Food Engineering 73: 313–319.

Wichienchot, S. 2006. Production of oligodraxtran by Gluconobacter oxydans NCIMB 4943 and evaluation on their prebiotic properties. Hat Yai, Thailand: Prince of Songkla University, PhD. thesis.

Wichientchot, S., Jatupornpipat, M. and Rastall, R. A. 2010. Oligosaccharides of pitaya (dragon fruit) pulp and their prebiotic properties. Food Chemistry 120: 850-857.