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Original Article

Extraction and analysis of prebiotics from selected plants from southern Thailand

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Abstract

Thirteen plants and their parts acquired from southern Thailand were investigated for their polysaccharide contents and prebiotic properties. The fresh, ground samples were extracted with 50% and 95% ethanol and water at ambient and boiling temperatures. The extracts were freeze-dried, digested with HCl buffer and α-amylase, and indigestible polysaccharide contents were determined. Base on extract yields and indigestible polysaccharide contents, ten samples were chosen as potential sources of prebiotics. These included embryo, flesh and pericarp of palm fruit (*Borrassus flabellifer* L.), skin, flesh and seed of jackfruit (*Artocarpus heterophyllus* Lam.), flesh of rambutan (*Nephelium lappaceum* L.), jampadah (*Artocarpus integer* Merr.), and young coconut (*Cocos nucifera* Linn.), and okra pod (*Hibiscus esculentus* Linn.). Their extract yields (% dry wt) were 26.54, 44.94, 51.69, 71.54, 59.43, 16.00, 55.73, 34.11, 22.66, and 12.39, respectively, and indigestible polysaccharide contents (mg/g dry extract) were 409.85, 334.87, 705.80, 689.08, 605.76, 403.44, 566.83, 542.56, 513.87, and 460.73, respectively. The amounts of oligosaccharide were 33.69, 47.20, 14.13, 0.00, 98.05, 29.35, 9.43, 2.40, 0.00, and 49.15 mg/g dry extract, respectively. Subsequently, five samples were chosen for further studies and possible commercial development based on their extract yield, the amount and type of oligosaccharides, i.e. palm flesh, palm embryo, jackfruit flesh, jackfruit seed, and okra pod. Molecular weights of the polysaccharides from the five samples were 190-1,600 Daltons with a degree of polymerization of 5-6.

Keywords: prebiotic, indigestible polysaccharides, southern Thailand plants, extraction

1. Introduction

Nutraceuticals and functional foods (NFF) are increasing in popularity as a tool of the consumers for the management of their health and wellness. Pre-, pro- and synbiotics are an important group of NFF shown to be effective in modulating gastrointestinal diseases and other ailments. Prebiotics are polysaccharides and oligosaccharides that can withstand digestion and absorption in the small intestine, but can be selectively fermented by probiotic bacteria native to the large intestine. They consist of short-chain carbohydrates, principally oligosaccharides, e.g. fructooligo-

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saccharides (FOS), galactooligosaccharides (GOS), and polysaccharide, e.g. inulin (Gibson, 1995; Panitantum, 2004). The colonic fermentation of prebiotics enhances the growth of probiotics, such as Bifidobacteria, Lactobacilli, and Eubacteria (Gibson and Roberfroid, 1995; Cummings *et al.*, 2001), but not of pathogens, such as *Clostridium perfringens*, which cause gastrointestinal diseases (Kolida *et al.*, 2002). Gibson *et al.* (1995) fed 5-20 g/d of FOS and inulin to 100 volunteers for nine days and found an increase in *Bifidobacterium*. The fermentation of prebiotics by the probiotic bacteria improves the host's health by enhancing the absorption of minerals such as Ca, Mg, and Fe and producing compounds capable of preventing colon cancer (Gibson and Roberfroid, 1995). During the fermentation, a large quantity of acids is produced, including short-chain fatty acids, resulting in the decrease of pH and possible reduction in the numbers of pathogenic microorganisms (Morisse *et al.*, 1993). Gastrointestinal physiology is also affected by these activities, which may benefit human health (Van Loo *et al.*, 1999).

Prebiotics occur naturally in fruits and vegetables, e.g. asparagus, onion, cereals, garlic, Jerusalem artichoke, chicory, banana, and small amounts are found in the forms of free sugars or glycoconjugates in human milk and animal colostrums (Bucke and Rastall, 1990). Recently, dragon flesh has been reported to be a source of oligosaccharides, which is a candidate prebiotic (Wichienchot *et al.*, 2010). Other plant constituents as candidates for prebiotics, for examples, are resistant starch, non-starch polysaccharides e.g. pectin, cellulose, hemicellulose, gums, and xylan. Prebiotics may also be synthesized from polysaccharides, such as starch or sugars, using appropriate enzymes; e.g. GOS may be synthesized from lactose with β-galactosidase; FOS may be produced from polymerization of fructose monomers by fructosyltransferase; isomaltooligosaccharide may be produced by transglucosylation of liquefied starch; whereas lactulose may be obtained from chemically synthesized by isomerization of lactose (Vernazza *et al.*, 2006). However, consumer's preference towards natural products or ingredients has encouraged the continuing search for viable natural sources for prebiotics. This paper describes a study on 13 fruits and vegetables and their parts, all found and acquired in southern Thailand, as potential sources of natural prebiotics.

2. Materials and Methods

2.1 Selection of plants

Thirteen types of fruits and vegetables commonly grown and consumed in southern Thailand were selected based on their physicochemical characteristics, which suggested the presence of prebiotics or soluble dietary fiber, abundance, low cost, and the amount of waste they generated that could be utilized. Table 1 lists the plants and their parts used in the studies.

2.2 Extraction of prebiotics from plant samples

The plant materials were purchased from a local fresh market, cleaned, and separated into various parts as indicated in Table 1. The samples were chopped and finely ground in a Waring blender. The ground samples were analyzed for moisture content (AOAC, 2000) and were then extracted with various solvents as indicated in Table 2.

Ethanol extraction procedure was repeated twice with fresh solvent on the same samples, while the water extraction was done only once since prolonged soaking in cold water would result in microbial growth. All extractions were done in triplicates. After the extraction was terminated the samples were filtered through linen filter cloth. The filtrates from the three extractions with ethanol were combined and all filtrates were concentrated in a rotary evaporator and freeze-dried. The freeze-dried samples were kept in capped dark glass bottles at -20 °C till use. The extract yields were calculated from:

Yield (% fresh weight) =
(weight dried sample (g) x 100) / weight fresh sample (g)

2.3 Determination of prebiotic properties of the extracts

2.3.1 Resistance to acidic and enzymatic digestion

Dried extracts were made into 10% solutions (w/v) with distilled water. For acidic digestion, each was incubated at 37°C with HCl buffer at pH 1 for 4 hrs according to the modified procedure of Korakli *et al.* (2002). The reaction was terminated with 1 N NaOH. For enzymatic digestion, the acid-digested solutions were further incubated at 37°C with 2 unit/mL human pancreatic α-amylase in phosphate buffer solution (20 mM) at pH 6.9 for 6 hrs according to Doyle *et al.* (1999) as cited in Wichienchot (2005). The enzymatic digestion was terminated by heating at 80°C for 10 min. All digestions were done in triplicates. To determine the amounts of indigestible polysaccharides in the extracts, the extracts were first analyzed for their reducing sugar contents (mg/g) using the modified dinitrosalicylic acid method (Miller, 1959). The digesta were then analyzed for total sugar contents (mg/g) with the modified phenol sulfuric method (Fox and Robyte, 1991). The indigestible polysaccharide content (mg/g dry extract) in the extracts was calculated from:

Indigestible polysaccharides (mg/g) =
Total sugar after acid-enzyme digestions (mg/g)
– Reducing sugar before the digestions (mg/g)

Based on the extract yields and indigestible polysaccharides in the extracts, ten samples were chosen for further studies. These included palm pericarp, jackfruit skin, jackfruit flesh, rambutan flesh, jampadah flesh, young coconut flesh, okra pod, palm embryo, jackfruit seed, and palm flesh.

Table 1. Plants and their various parts used in the analysis for prebiotics.

Plant	Parts Used in the Studies
1. Banana (*Musa sapientum* Linn.; Thai name: Kluay Naam Wah), a. mature green, b. ripe	a. Skin b. Flesh
2. Okra (*Hibiscus esculentus* Linn.; Thai name: Krajiab Kheaw), mature green	a. Pod
3. Jackfruit (*Artocarpus heterophyllus* Lam.; Thai name: Ka Nhoon), ripe	a. Skin (inner rind, i.e. excluding the outer skin) b. Flesh c. Seed
4. Germinated brown rice (*Oryza sativa* Linn.; Thai name: Kao Ngog)	a. Germinated grain
5. Rambutan (*Nephelium lappaceum* Linn.; Thai name: Ngoh), ripe	a. Peel b. Flesh c. Seed
6. Durian (*Durio zibethinus* Linn.; Thai name: Turian), ripe	a. Shell
7. Jampadah (*Artocarpus integer* Merr.), ripe	a. Skin b. Flesh c. Seed
8. Huasa potato (*Coleus parvifolius* Benth; Thai name: Man Kee Nhoo), mature	a. Tuber
9. Tamarind (*Tamarindus indica* Linn.; Thai name: Ma Kham), ripe	a. Flesh b. Seed coat c. Seed cotyledon
10. Coconut (*Cocos nucifera* Linn.; Thai name: Ma Prao), young fruit	a. Flesh
11. Mango (*Mangifera indica* Linn.; Thai name: Ma Muang), mature green	a. Skin b. Flesh c. Seed
12. Fan palm fruit (*Borassus flabellifer* Linn.; Thai name: Luke Taan)	a. Pericarp of ripe fruit b. Flesh from young fruit c. Embryo
13. Dioscorea tuber (*Dioscorea membranacea* Pierre; Thai name: Hua Khao Yen)	a. Tuber

Table 2. Solvents and extraction procedures used for each sample.

Solvent	Proportion of Solvent to Sample	Temperature	Time
Ethanol (EtOH), 95%	Complete cover of sample	Ambient, ~ 30 °C	3 days
Ethanol (EtOH), 50%	Complete cover of sample	Ambient, ~ 30 °C	3 days
Water (CW)	Complete cover of sample	Ambient, ~ 30 °C	4 hrs
Water (HW)	Complete cover of sample	Boiling (100 °C)	15 min

2.3.2 Analysis of sugars in the extracts with High Performance Liquid Chromatography (HPLC)

Qualitative and quantitative analyses of the sugars in the ten chosen extracts were performed with HPLC, using the modified methods of Tieking *et al.* (2005) and Schwab and Ganzle (2006). The 10% solutions of the extracts were diluted four times and 20 µL diluted samples were injected into the HPLC to determine the amount of mono- and disaccharides in the extracts. The same 10% solutions were digested at 80°C for 1 hr with H_2SO_4 at the final concentration of 2 N and terminated by neutralizing it with 1 N NaOH. The digesta were filtered through a 0.2 µm filter medium and diluted four times, and 20 mL of the diluted filtrates were injected into the

HPLC. The HPLC used was Shimadzu CR6A Chromatopac, Japan, with column Agilent Zorbax LC-NH_2, 4.6 mm x 250 mm, 5 µm. The mobile phase was acetonitrile:water at 75:25, the flow rate of 1 mL/min, ambient temperature, and RI detector. The standard sugars used were D-glucose, D-fructose, and sucrose. All analyses were done in triplicates. The amounts of indigestible polysaccharides in the extracts determined by the HPLC method were calculated from:

Indigestible polysaccharides (mg/g dry extract) =
Total sugars after H_2SO_4 digestion (mg/g dry extract) – Sugars before digestion (mg/g dry extract).

Based on the amounts of indigestible polysaccharides in the extracts by the HPLC method, five samples from the ten samples previously chosen were selected for further studies. These included palm flesh, palm embryo, jackfruit flesh, jackfruit seed, and okra pod.

2.3.3 Determination of molecular weight distribution in the extracts

The final five selected extracts were analyzed for the molecular weights (MW) distribution. Monosaccharides were first removed from the extracts by precipitating them with 80% ethanol (EtOH) twice. The samples were then sent to the Material Technology Center, a division of the National Science and Technology Development Agency in Bangkok, Thailand, for the MW analysis. The method used was gel permeation chromatography (GPC). The freeze-dried extracts were dissolved in 0.1 M $NaNO_3$ to the concentration of 0.1% (w/v). The solutions were filtered through 0.2 mm nylon syringe filter before 20 µL samples were injected into the GPC (Polymer Laboratories, England). The column used was Ultrahydrogel Linear (Water, USA), at the column temperature of 30°C, flow rate of 0.6 mL/min, with RI detector. Pullulans were used as standards for MW comparison, and PL Logical GPC software (England) was used to analyze the results.

3. Results and Discussion

3.1 Extraction of polysaccharides from plant samples

The original number of samples studied was 28, taken from 13 plants and their various parts. Each was extracted with four solvents, i.e. water at ambient temperature (CW), boiling water (HW), 50% EtOH and 95% EtOH, each extraction was done in triplicates and average results ± SD were reported. Except for a few samples, EtOH, either 50 or 95%, was more effective as a solvent than CW or HW. This is most likely due to the fact that the extraction was done over three days and each sample was extracted three times and the three extracts were combined, while the CW and HW extractions were done only once over 4 hrs and 15 min, respectively, to avoid possible microbial problems.

In order to find ways to improve the efficiency of the extraction, a preliminary study was conducted on a few selected samples to determine the effects of particle size, sample to solvent ratio, agitation, temperature, and extraction time on extract yield. Results show that samples varied in their responses to these variables due to their physical and chemical characteristics. Most samples became viscous pulps if ground too fine and for some, e.g. okra pod, it was impossible to extract something as it became a gelatinous mass when mixed with the solvents. However, a general conclusion could be drawn that the following extraction conditions would improve the yield of most of the samples: particle size of 5 mm diameter; sample to solvent ratio of 1:2; agitation at 200 rpm; room temperature; and 60–150 min extraction time. These conditions are being optimized in batch and continuous pilot-scale extractors (to be reported in a separate paper).

3.2 Determination of indigestible polysaccharides in the extracts

Gibson and Roberfroid (1995) defined a prebiotic as *"a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon and thus improves host health*". Prebiotics must be able to withstand digestive processes prior to reaching the colon to have these effects (Gibson *et al.*, 2004). Digestion in the stomach occurs under a very acidic condition (pH 1-3) and involves various enzymes secreted by pancreas (Rowland and Mallett, 1990; Macfarlane and McBain, 1999) one of which is α-amylase. Therefore, in the initial evaluation of the prebiotic properties of the extracts the crude extracts were subjected to acidic and then enzymatic hydrolyses using HCl buffer and α-amylase, respectively, to determine the amounts of their indigestible polysaccharides. However, the "prebiotics" in the crude plant extracts are very complex and may include short- and long-chain oligosaccharides and non-starch polysaccharides, such as pectin, cellulose, hemicellulose, and xylans (Macfarlane *et al.*, 1992). Therefore, to determine the amounts of oligosaccharides in the crude extracts, the indigestible oligosaccharides were subsequently analyzed using the H_2SO_4 digestion method. The results of the two analyses of the indigestible polysaccharides, i.e. acid/enzyme-digested and H_2SO_4-digested, are presented in Table 3.

Results in Table 3 indicate that various parts of palm fruit and jackfruit appear to have a high potential for further development as commercial prebiotics due to their high extract yields and high concentrations of indigestible polysaccharides. Unfortunately, some of them while having high extract yield and total indigestible polysaccharides have low or no oligosaccharides, e.g. jackfruit skin, jampadah skin, and young coconut flesh with 0, 2.40, and 0 mg oligosaccharides/g dry extract, respectively. Okra pod has the second highest oligosaccharide concentration (49.15 mg/g) and medium range total indigestible polysaccharides (460.73 mg/g) among the ten selected samples, but its extract yield is the lowest

Table 3. Moisture content, extract yield, and indigestible polysaccharide concentrations in ten Thai plant samples.

Plant	Part	Moisture content (% wet weight±SD)	Solvent	Extract yield (% dry weight±SD)	Indigestible polysaccharides, acid/enzyme digestion[a] (mg/g dry extract±SD)	Indigestible polysaccharides, H_2SO_4 digestion[b] (oligosaccharides) (mg/g dry extract)
Palm fruit	Pericarp	85.53±0.10	EtOH 95%	51.69±0.40	705.80±3.83	14.13
Jackfruit	Skin	88.65±0.03	EtOH 95%	71.54±0.01	689.08±15.21	0.00
Jackfruit	Flesh	81.76±0.01	EtOH 95%	59.43±0.07	605.76±16.55	98.05
Rambutan	Flesh	82.81±0.07	EtOH 50%	55.73±2.65	566.83±8.42	9.43
Jampadah	Flesh	69.83±0.06	EtOH 95%	34.11±0.12	542.56±13.82	2.40
Young coconut	Flesh	86.45±0.06	CW	22.66±5.59	513.87±4.29	0.00
Okra	Pod	89.99±0.03	EtOH 50%	12.39±0.01	460.73±17.05	49.15
Palm fruit	Embryo	77.66±0.20	EtOH 50%	26.54±0.96	409.85±2.88	33.69
Jackfruit	Seed	71.50±0.20	EtOH 50%	16.00±0.08	403.44±5.63	29.35
Palm fruit	Flesh	91.99±0.04	EtOH 95%	44.94±0.26	334.87±19.45	47.20

Note: SD = standard deviation; EtOH = ethanol, CW = water at ambient temperature. [a] SD was calculated from averaged SDs of total sugars and reducing sugars. [b] Due to the calculation method used it was not practical to calculate SD.

(12.39% dry weight). Unless its extract yield can be significantly improved through modifications of the extraction procedure or it can be processed into other forms, e.g. dried powder, its commercial potential is doubtful. Therefore, samples showing high potential for further research and development are jackfruit flesh, palm flesh, palm embryo, and okra pod. Jackfruit seed should also be considered, though its extract yield is quite low, since it is a waste product and should be very cheap. Rambutan flesh is another potential candidate prebiotic since the fruit currently has low market value.

The marked difference between the total indigestible polysaccharides (acid and enzyme-digested) and oligosaccharides (H_2SO_4-digested) indicates that the indigestible carbohydrates in these ten samples consist mainly of non-starch polysaccharides, e.g. pectin, cellulose, hemicellulose, and xylans. These non-starch polysaccharides also are prebiotics candidates since they can be fermented by the colonic microflora (Bucke and Rastall, 1990).

Some of these prebiotics candidates were further studied on their selectively fermentation in artificial colon system by colonic microflora under anaerobic condition, 37°C and controlled pH at 5.5±0.1, 6.2±0.1, and 6.8±0.1 for proximal, transverse, and distal regions, respectively. Oligosaccharides of jackfruit flesh and seed were confirmed on their prebiotic properties since they had selectively fermentation by lactobacilli and produced significantly amount of butyric acid (to be reported in a separate paper).

Analysis of sugars in the ten extracts with HPLC, prior to H_2SO_4 digestion, revealed that they consisted mainly of two types of monosaccharide, i.e. glucose and fructose, and one type of disaccharide, i.e. sucrose. Rambutan flesh extracted with 50% EtOH contained the highest amount of sugars at 783.22 mg/g dry extract, followed by jackfruit flesh extracted with 95% EtOH at 733.11 mg/g, jampadah flesh extracted with 95% EtOH at 684.54 mg/g, while young coconut flesh extracted with CW having the lowest amount of 127.56 mg/g. Jackfruit seed extracted with 50% EtOH contained 331.45 mg/g; palm flesh with 95% EtOH, 487.68 mg/g; palm pericarp with 95% EtOH, 416.22 mg/g; palm embryo with 50% EtOH, 503.92 mg/g, and okra pod with 50% EtOH, 332.36 mg/g dry extract.

3.3 Molecular weight determination of prebiotics

Taking extract yield, total indigestible polysaccharide content, oligosaccharide content, and the results of probiotic fermentation (to be reported in a separate paper) into account, five samples were chosen for further studies and possible commercial development. These included palm flesh, palm embryo, jackfruit flesh, jackfruit seed, and okra pod. Molecular weights (MW) of their oligosaccharides were determined, using the GPC method. Average MWs of the five samples were quite similar, being 190–1,600 Daltons. If mono- and disaccharides excluded, the oligosaccharides contents of each sample were as follow. The MW distributions of palm flesh oligosaccharides were 508, 989, and 1,587 Daltons; palm embryo oligosaccharides were 465, 834, and 1,557 Daltons; jackfruit flesh and seed oligosaccharide were 1,116, 461, and 1,080 Daltons, respectively, and okra oligosaccharides were 850 and 1,512 Daltons.

The principal oligosaccharides in palm flesh and embryo have the degree of polymerization (DP) of 5, while that of jackfruit flesh was 6, and of jackfruit seed and okra pod were 5. This appears to indicate that the majority of the indigestible carbohydrates in these plants are of oligosaccharides with medium chain lengths.

4. Conclusion

Several plants cultivated in southern Thailand appear to contain considerable amounts of polysaccharides that are prebiotics candidates since they can withstand the artificial acidic conditions in stomach and human pancreatic and α-amylase in the small intestine. Among the 28 parts of 13 plants, five appear to have the highest potential for further research and development and commercialization based on extract yield and the amount and type of indigestible oligosaccharides. These include palm flesh, palm embryo, jackfruit flesh, jackfruit seed, and okra pod. At least two of them, jackfruit flesh and seed, were confirmed on their prebiotic property by selectively *in vitro* colonic microflora fermentation in an artificial colon system. The MW distributions of the five extracts ranges between 190 and 1,600 Daltons; the majority of which have medium chain length with the degree of polymerization between 5 and 6.

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