https://doi.org/10.48048/tis.2023.6770

Potential Prebiotic Properties of Crude Polysaccharide Extract from Durian (*Durio zibethinus* Murr.) Seed Flour

Patthinan Varichanan[1,*], Sirinan Shompoosang[1] and Sawitree Dueramae[2]

[1]Department of Applied Microbiology, Institute of Food Research and Product Development, Kasetsart University, Bangkok 10900, Thailand
[2]Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand

([*]Corresponding author's e-mail: ifrptn@ku.ac.th)

Received: 21 February 2023, Revised: 10 April 2023, Accepted: 18 April 2023, Published: 28 August 2023

Abstract

The purpose of this research is to investigate the possible presence of prebiotic substances in durian seeds. The durian seed flour (DSF) was prepared by drum dry processing at 140 °C. The proximate composition of DSF 100 g contained moisture (2.43 g), protein (6.85 g), total fat (0.60 g), total carbohydrate (87.73 g), ash (2.39 g), and the sum of dietary fiber (4.48 g), respectively. The morphology of DSF was studied by SEM at a fixed magnification (100×). DSF appears to have smooth and rough surfaces, and the granules are covered by the gum. The optimal condition for extracting crude polysaccharide from DSF is ethanol 50 % at solvent ratios 1:8 at a temperature of 30 °C for 90 min, gave a maximum extraction yield of 5.28 % and the maximum amount of non-reducing sugar content at 392.19 ± 10.60 mg mL^{-1}. The amount of non-reducing sugar is represented by an oligosaccharide referred to as a prebiotic. After being digested *in vitro*, the crude polysaccharide extract and inulin each had a total percentage of hydrolysis that was 14.68 and 11.54 %, respectively. Thus, approximately 85.32 % of crude polysaccharide extract was estimated to reach the colon. Moreover, the crude polysaccharide extract has enhanced activity values higher than inulin with 3 probiotic strains. The enhanced activity value increases when the extract concentration increases. The concentration of crude polysaccharide extract was 5.0 %w/v. *Lactobacillus rhamnosus* TBRC 374 had the highest enhanced activity at 582.67 %. In comparison, *Lactobacillus casei* BCC 13300 and *Lactobacillus acidophilus* ATCC 4356 with enhanced activity were 543.78 and 288.34 %, respectively. Significant advancements have been made in the utilization of plant waste and the development of novel prebiotic ingredients with the potential for application in food as a result of this research.

Keywords: Durian seed flour, Enhance activity, *In vitro* digestion, Non-reducing sugar, Polysaccharide extraction, Prebiotic, Probiotic bacteria

Introduction

In the modern period of globalization, human cultures have become more urbanized, and manufacturing dominates economic activity over agriculture. This phenomenon profoundly influences the human way of life, food, and physical activity. Their way of life necessitates less physical exertion. This modern lifestyle is more likely to affect the diversity of the human gut microbiota, changing its microbial composition and increasing the risk of a wide variety of chronic and non-communicable diseases (NCDs) such as type 2 diabetes, cardiovascular disease, and hypertension [1]. Prebiotics could be utilized as a potential solution for this problem. Numerous research has proved the clinical benefit of prebiotics in improving human health by increasing the number of gut bacteria. The International Scientific Association for Probiotics and Prebiotics defines prebiotics as "substances selectively exploited by host bacteria that give health advantages" [2]. A well-known probiotic is the host microorganism, a chosen and live bacterium that, when ingested in appropriate amounts, has a favorable effect on human organisms via its intestinal tract effects [3].

Prebiotics are frequently obtained using 3 methods: Microbiological synthesis, polysaccharide enzymatic degradation, and isolation from natural resources [4]. Three criteria must be satisfied for a food component to be classified as a prebiotic. First, the food must be resistant to gastric acidity, mammalian enzyme digestion, and intestinal absorption. Second, the food must be fermentable by the bacteria in the digestive tract. Thirdly, it must stimulate preferentially the growth and activity of beneficial bacteria in the intestines of mammals, including humans [5]. Prebiotics has a significant impact on human health.

Asparagus, banana, barley, beans, chicory, garlic, honey, Jerusalem artichoke, onion, peas, sugar beet, wheat, tomato, rye, soybean, human's, and cow's milk, etc., and more recently, seaweeds and microalgae contain prebiotics naturally [6]. Recently, Artocarpus integer's seed has been reported to be a source prebiotic [7]. Other plant constituents as candidates for prebiotics, for example, are resistant starch and non-starch polysaccharides.

Recently, there has been an increase in interest in researching, identifying, and producing novel prebiotic substances to support the argument that functional foods could be utilized as an alternative strategy for boosting well-being and lowering the risk of disease [8,9].

In these perspectives, Durian (*Durio zibethinus* Murr.) is the most popular tropical fruit in South-East Asia. In 2018, Thailand had a total of 937,607 rai of durian plantations, and durian production was 655,362 tons. Durian paste is a product of durian that has produced a volume of 1,211 tons with a value of 131.7 million Thai Baht [10]. Southern Thailand uses local durian as a material to make durian paste because it has a large seed, less flesh, and a strong smell; therefore, it is not customarily consumed fresh. In the processing of durian paste, the seeds are discarded. According to [11], durian seeds are wasted to the environment 75,000 tonnes per year-which will continue to rise as much as there's a demand for durians worldwide. However, they could be processed into durian seed flour (DSF) to increase the use-value of durian waste. A great resource of functional food ingredients, especially prebiotics, is one that is high in quantity of carbohydrates, particularly non-digestible carbohydrates.

Durian seeds consist primarily of mucilage (gum) and starch. Numerous investigations have been conducted on durian seed starch and flour. According to [12], durian seed starch contains approximately 23 % amylose and 5 % resistant starch [13], also reported that durian seed flour (DSF) contained amylose amylopectin about 22.35 and 66.33 %, respectively. Amylose in DSF is important in gel formation, while amylopectin is associated with the sticky property [14]. The yield of durian seed gum following aqueous extraction is approximately 56 % of the dry seed flour [15]. The gum in DSF affects its characteristics as a hydrocolloid consisting of polysaccharides and proteins [16]. The previous study, chemical extraction was performed and indicated that DSF contains polysaccharides that might be prebiotic properties [16]. Therefore, the polysaccharide extraction from DSF is attractive in the prebiotic properties of Durian seeds. In this study, Durian seed flour was prepared by drum dry processing that can maintain fiber and texture, suitable for application in food industry. Moreover, the utilization of such waste is necessary for the cultivation of durian and the increase of income in this industry, particularly if the product in question has the prospect to serve as a functional food ingredient in countries in which the durian is cultivated, such as countries in Southeast Asia.

The objectives of the present study were to optimize the extraction method of polysaccharides from durian seed flour (DSF) and analyze their prebiotics as resistant ability to *in vitro* digestion, including stimulating the growth of probiotics include *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus rhamnosus* TBRC 374, and *Lactobacillus casei* BCC 13300. Because Lactobacilli are ordinarily present in the gastrointestinal tract, they are commonly used as representative groups of probiotics, which the Thai FDA allows as functional ingredients in foods.

Materials and methods

Chemicals

Analytical grade was employed for all of the chemicals, solvents, and medium components that were utilized in this work. The bovine bile and porcine pancreatic beta-amylase were both purchased from Sigma-Aldrich in the United States. Merck (Darmstadt, Germany) was the supplier for the sodium chloride (NaCl), sodium hydroxide (NaOH), and hydrochloric acid (HCl) that were obtained. Calcium chloride dihydrate ($CaCl_2.2H_2O$) sodium dihydrogen phosphate (NaH_2PO4) magnesium chloride hexahydrate ($MgCl_2.6H_2O$) magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) and iron (II) chloride 4-hydrate ($FeCl_2.4H_2O$) were purchased from Ajax Finechem (Taren Point, NSW, Australia).

Bacterial strain used

The probiotic strain *Lactobacillus acidophilus* ATCC 4356 was obtained American Type Culture Collection. *Lactobacillus rhamnosus* TBRC 374, and *Lactobacillus casei* BCC 13300 were purchased from National Center for Genetic Engineering and Biotechnology (BIOTECH), Pathum Thani, Thailand. All probiotic strains were stored at −20 °C for further study. Lyophilized probiotic strain cultures were rehydrated in nutrient broth at 37 °C for 24 h. To prepare the inoculum, 1 mL of the rehydrated culture was grown in MRS broth at 37 °C for 24 h.

Durian seed sample

Local durian (*Durio zibethinas* Murr.) seeds were obtained from the residue of local durian paste production in Sako, Sungai Padi district, Narathiwat province, southern Thailand. Local durian seeds have large seed sizes of about 4 - 6 cm in length and 3 - 4 cm in diameter.

Preparation of durian seed flour by twin drum dryer

The manufacture of durian seed flour is depicted in **Figure 1**. Water was applied to clean durian seeds, and the seeds were boiled in the ratio of seed (kg): Hot water (L) about 1:3 at 100 °C 30 min. By removing the brown seed covering, the cooked durian seeds were dehulled. After peeling, the dehulled durian seeds were mixed with water in a ratio of 1:1 (w/v), and the mixture was well blended for 3 min or until smooth. The liquid durian seed flour was dried at 140 °C by a twin drum dryer. The drum dryer speed and gap were set at 7 rpm and 0.4 mm. After drum drying, the moisture content of the durian seed flour sample was ≤ 8 %. The durian flour sample was packaged in vacuum-sealed polyethylene bags and kept at room temperature until further analysis.

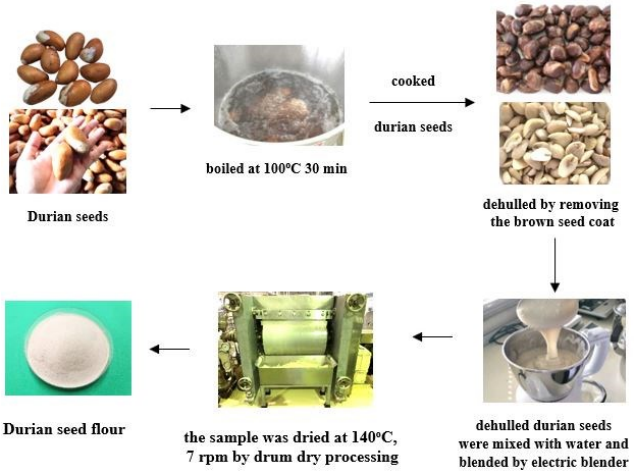

Figure 1 Process of durian seed flour preparation.

Determination of physical properties

The color of the durian seed flour was determined using a digital color analyzer (Hunter Lab Model Color Flex EZ, United States), and the results were reported as L^*, a^*, and b^*. L^* symbolizes lightness, a^* indicates chromaticity from green (−) to red (+), and b^* indicates chromaticity from blue (−) to yellow (+) [17].

Water solubility index (WSI) and water absorption index (WAI) were analyzed using the method according to Anderson *et al.* [18]. The durian seed flour of 2.5 g was added to 50 mL centrifuge tubes containing 30 mL of distilled water and was suspended at room temperature for 30 min with gentle intermittent stirring. After that, the solution was centrifuged at 2,200 rpm for 15 min. The supernatant was added in the aluminum foil and was dried at 105 °C. After drying, the samples were determined up to constant weight. The WSI, which is the weight of dry soluble solids in the supernatant, is represented as a percentage of the sample's initial mass. WAI, the weight of gel obtained after supernatant removal, is represented as the weight of gel obtained per gram of extrudate. The WSI and WAI can be calculated from the following equations;

WSI = (weight of supernatant / weight of sample)×100

WAI = (weight of pellet in centrifuge tube - weight of centrifuge tube)/weight of sample

Determination of proximate composition

The official AOAC methods [19], were used to determine the moisture content (AOAC 931.04), crude protein (AOAC 991.20), total fat (AOAC 922.06), total dietary fiber (AOAC 985.29), soluble dietary fiber (AOAC 993.19), insoluble dietary fiber (AOAC 991.42), fructan (AOAC 997.08, inter 2000 83 (4)), and ash content (AOAC 930.30). The total carbohydrate content was estimated by subtracting 100 from the sum of the protein content, total fat content, moisture content, and ash content [20].

Granule morphology

Utilizing scanning electron microscopy (SEM, FEI, Quanta-400), the morphology of starch granules was examined. Gold granules were sputter-coated. At a 100× acceleration voltage, images of starch granules were acquired [21].

Optimization of extraction method

In this study, different solvent extraction (distilled water, EtOH 50 %, and EtOH 95 %) were used to evaluate of their effect on crude polysaccharide extract. The durian seed flour was extracted the crude polysaccharide extract by varying the ratio of flour: Solvent (w/v) about 1:2, 1:4, 1:8, and 1:16. The mixtures were extracted at 30 and 60 °C for 90 min. After that, all the solutions were filtered with a filter cloth. The filtrated samples were centrifuged at 3,000 rpm for 3 min. After centrifugation, the supernatants were evaporated at 25 °C for 6 h using the rotary evaporator (Rotavapor r-215, Buchi corporation co Ltd, USA). The crude polysaccharide extract was freeze-dried (Telstar Lioalfa-6) at −40 °C, 0.10 mBar for 48 h. After being freeze-dried, the samples were ground into a powder using a grinder before being placed in a plastic tube, sealed with a cap, and wrapped in aluminum foil to be stored at a temperature of −20 °C until use. The yield of extract was calculated from the following Eq. (1);

$$\text{Yield (\% extract)} = (\text{weight dried sample (g)}/\text{weight fresh sample}) \times 100 \quad (1)$$

The total sugar content was determined by the reaction of sugars with phenol in the presence of sulfuric acid using glucose as a standard [22]. The reducing sugar content was determined by the modified dinitrosalicylic acid method using glucose as a standard [23,24] and non-reducing sugars were calculated as the difference between Total sugar and reducing sugars using the following Eq. (2) [25];

$$\text{Non-reducing sugar} = \text{Total sugar} - \text{reducing sugar} \quad (2)$$

Determination of total sugar of extracts

Using the phenol-sulfuric acid assay method published by Dubois *et al.* [22], the total sugar content of an extract that had performed acid and enzyme hydrolysis was measured. The assay was calibrated using D-glucose standards between 0 and 100 g mL^{-1}. One mL of sample extract or glucose standard solution was added to 1 mL of a 5 % phenol solution. Then, 5 mL of concentrated sulfuric acid was added to the combination of sample glucose and phenol, which was left to react for 10 min prior to vigorous mixing and measuring the absorbance at 490 nm (Thermo scientific Genesys UV/Vis Spectrophotometer, USA). The total sugar content of the extract was measured by utilizing the measurements of standard D-glucose from the standard curve.

Determination of reducing sugar of extracts

Following acid hydrolysis and enzyme hydrolysis, the reducing sugar content of the extract was calculated using a modified version of the DNS assay reported by Miller [23], and Robertson *et al.* [24]. For the calibration of the assay, a D-glucose standard solution with a concentration of 0 - 1 mg mL^{-1} was produced. One milliliter of the sample extract or glucose standard solution was added to 3 milliliters of DNS solution and 1 milliliter of distilled water. The mixture was cooked in a boiling water bath for 5 min before being cooled to room temperature. After cooling, 5 mL of pure water was added, and the absorbance at 540 nm was measured using a Thermo scientific Genesys UV/Vis Spectrophotometer (America). The amount of reducing sugar in the sample was evaluated by comparing the values of the extract to those of standard D-glucose from the standard curve.

***In vitro* digestion studies**

Digestion by artificial enzymatic in mouth

Human salivary α-amylase (Sigma A1031) was used to perform *in vitro* enzymatic digestions of extracted materials compared to an inulin standard as a commercial prebiotic reference (positive control) [26]. The enzyme 0.3 U mL^{-1} was prepared in 20 mM sodium phosphate buffer (pH 6.8, Na_2HPO_4 1.42 g L^{-1}, KH_2PO_4 1.36 g L^{-1}, NaCl 0.58 g L^{-1}). 5 mL of enzyme solution was combined with 5 g of extracted sample. The combinations were incubated in a water bath at 37 °C for 60 min. After incubation, 1 mL of the mixture was extracted and analyzed for the final reducing sugar level. As a positive control, an inulin solution (1 %w/v) was utilized. The total sugar concentration and total reducing sugar content of the extracted sample before digestion were also determined. Eq. (3) was used to compute the percentage of hydrolysis of the extracted sample.

$$\%\text{hydrolysis} = \frac{\text{final reducing sugar content} - \text{initial reducing sugar content}}{\text{total sugar content} - \text{initial reducing sugar content}} \times 100 \quad (3)$$

Digestion by gastric juice in stomach

In order to simulate human stomach juice, the following compounds were suspended in 1 L of deionized water; 8.25 g of disodium phosphate dihydrate ($Na_2HPO_4.2H_2O$), 0.2 g of potassium chloride (KCl), 8 g of sodium chloride (NaCl), 0.1 g of calcium chloride dihydrate ($CaCl_2.2H_2O$), 14.35 g of sodium hydrogen phosphate ($NaHPO_4$), and 0.18 g of magnesium chloride hexahydrate ($MgCl_2.6H_2O$). Using 5 M hydrochloric acid (HCl), the gastric juice's acidity was brought down to pH 2 [7]. Five mL of simulated human stomach juices pH 2 were combined with 1 mL of the extracted sample from *in vitro* oral digestion, and the mixture was then incubated for 2 h at 37 °C in a water bath. Following incubation, 1 mL of the mixture was taken out and its final reducing sugar concentration was determined. As a positive control, 1 %(w/v) inulin solution was employed. Prior to the digestion process, the extracted sample's total and decreasing sugar contents were also identified. Eq. (3) was used to determine the percentage of hydrolysis of the sample that was extracted.

Digestion by artificial enzymatic in small intestine

Enzymatic digestions of extracted samples were carried out *in vitro* using porcine pancreatic α-amylase [26], and the results were compared to an inulin standard used as a commercial prebiotic reference (positive control). Ten mL porcine pancreatin was prepared in 0.5 mg mL^{-1} in $CaCl_2$ 25 mM, 12 g bovine bile at pH 6.9 and was mixed with 1 mL extracted sample from *in vitro* stomach digestion. The solutions were incubated in a 37 °C water bath for 2 h. After incubation, 1 mL of the solution was removed and the final reducing sugar content was determined. As a positive control, 1 %w/v inulin solution was employed. The total sugar concentration and total reducing sugar content of the extracted sample before digestion were also measured. Eq. (3) was used to calculate the percentage of hydrolysis of the extracted sample. The percentage of hydrolysis obtained from the mouth, stomach, and small intestine was calculated for the total percentage of hydrolysis as Eq. (4) [27].

$$\text{Total\%hydrolysis} = (\%\text{hydrolysis in mouth} + \%\text{hydrolysis in stomach} + \%\text{hydrolysis in small intestine}) \quad (4)$$

Enhanced activity of the crude polysaccharide extract from DSF on probiotic growth

The modified approach of Phothichitto *et al.* [28], was used to examine the enhanced activity of the extract on the growth of probiotics. The probiotic strain *Lactobacillus acidophilus* ATCC 4356*, Lactobacillus rhamnosus* TBRC 374, and *Lactobacillus casei* BCC 13300 were rehydrated in nutrient broth for 24 h at 37 °C. To prepare the inoculum, 1 mL of the rehydrated culture was cultured in MRS broth at 37 °C for 24 h using 1 mL of the culture. The extracted and positive prebiotic control (inulin) were used as the individual carbon sources for the growth of probiotics in MRS medium. After preparing 3 different concentrations of sample extract solution (1, 2, and 5 %w/v), 1 mL of each was added to 3 mL of MRS broth along with 1 mL of inoculum. In the controlled set, the sample extract solution was switched out for water that had been through the distillation process. The culture mixture was kept in an incubator at 37 °C for a period of 24 h. The total plate count on MRS agar after 24 h at 37 °C was used to quantify the number of live cells present in the bacterial cultures. Using Eq. (5), the percentage of enhanced activity of the extracted was estimated.

$$\text{Enhanced activity (\%)} = \frac{(SB - CB)}{CB} \times 100 \quad (5)$$

where SB is number of viable cells in MRS with extract (CFU mL^{-1}) and SC is number of viable cells in MRS without extract (CFU mL^{-1})

Statistical analysis

The significance level for the ANOVA and Post Hoc tests was 5 % ($p < 0.05$), indicating that the statistical analysis was 95 % certain to be accurate. The statistical study was performed with the statistical software IBM SPSS version 22.0 for Windows.

Results and discussion

Proximate composition and physical properties of durian seed flour

This study was carried out to determine the proximate composition and physical properties of durian seed flour (DSF) are presented in **Table 1**. The results revealed that DSF 100 g contained moisture 2.43 g, protein 6.85 g, total fat 0.60 g, total carbohydrate 87.73 g, ash 2.39 g, and the sum of dietary fiber 4.48 g, respectively.

In addition, the analysis of the amount of fructan (inulin + fructo-oligosaccharides), a substance in the group of prebiotics, found that in 100 g of durian seed flour, the amount of fructan is 20 mg. According to these results, the carbohydrates of DSF consist of starch and non-starch polysaccharides. Amid and Mirhosseini [15], recovered 56 % gum from durian seed flour in a previous study. The durian seed gum is a dietary fiber that is not broken down by digestive enzymes [29]. While the protein content is similar to the research of Permatarari *et al.* [30], that studied about nutritional of durian seed flour from west Kalimantan, Indonesia, it was found that the protein content was 6.2 %. The water activity at 0.03 and viscosity at 15 cps were found for the physical properties of durian seed flour. Water solubility and water absorption were 24.34 and 12.75 %, respectively. According to a previous report, it was found that 25 and 11 %, respectively [31]. These findings indicated that the non-starch constituents of DSF, specifically the mucilage, significantly contributed to water absorption [32]. The gum of durian seed is composed of protein-polysaccharide complexes and has the ability to absorb relatively significant quantities of water [15].

The particle size is mostly from 80 - 100 Mesh to 74.32 %, which follows the size of the sieve used in the sieving. **Figure 2** depicts, at a fixed magnification (100x), SEM images of the morphologies of DSF created using a twin-drum dryer. According to the SEM images, DSF appears to have smooth and rough surfaces, and the granules are covered by the gum. Due to the high drying roller temperature of 140 °C, water rapidly evaporates from the starch, resulting in a porous structure that enhances the solubility and absorption of DSF in water [33]. However, the morphologies of DSF depend on the temperature that heating; starch granules are gelatinized, disrupting of multiscale structures, with the extent of disruption depending on temperature, water content, and heating time rate of the method to preparation of durian seed flour [33,34].

Table 1 Proximate composition and physical properties of durian seed flour.

Parameter	Durian seed flour 100 g
Proximate composition	
Moisture (g)	2.43
Protein (g)	6.85
Total fat (g)	0.60
Total Carbohydrate (g)	87.73
Ash (g)	2.39
Dietary fiber	
Total dietary fiber (g)	4.46
Soluble dietary fiber (g)	2.78
Insoluble dietary fiber (g)	1.68
Fructan (g)	0.02
Sum of Dietary fiber (g)	4.48
Physical property	
Water activity (25 °C)	0.03
Viscosity (cps)	1.50×10^{1}
Particle size – Mesh No (%)	
< 40	7.27
40 - 60	0.56
60 - 80	2.05

Parameter	Durian seed flour 100 g
80 - 100	74.32
100 - 120	10.23
> 120	5.57
Color	
L^*	80.54
a^*	+1.80
b^*	+16.71
Water solubility (%)	24.34
Water absorption (%)	11.75

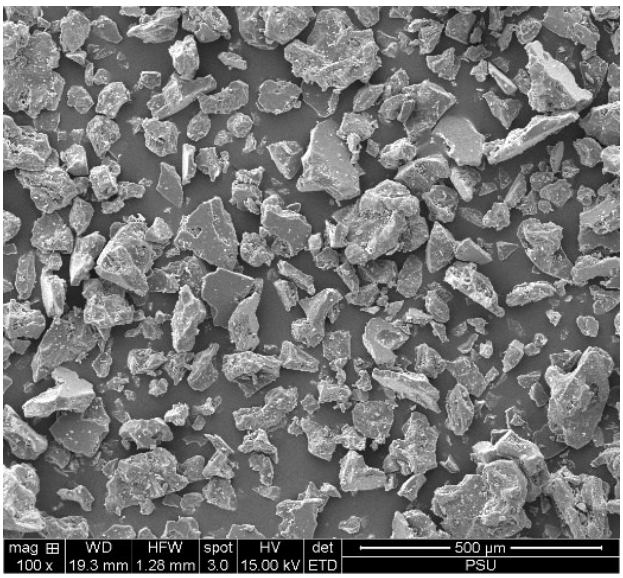

Figure 2 Morphologies of DSF by Scanning electron microscopy.

Optimization of extraction method

The experimental result of the optimum conditions for the crude polysaccharide extract from durian seed flour is in **Figure 3**. The ratio between durian seed flour and solvent at 1:8 gives the highest results in all treatments. It was found that ethanol at 50 % concentration gave the maximum yield of the crude polysaccharide extract (5.28 %) more than distilled water and ethanol at 95 % concentration at 30 °C. Due to durian seed flour composition consisting of protein at 6.85 %, the high concentrations of ethanol can affect protein denatured, which interferes with the diffusion of oligosaccharides resulting in incomplete extraction. Similar to Xiaoli *et al.* [35], study about the extraction of oligosaccharides from chickpeas, when the condition of extraction used at a concentration of ethanol more than 50 %, the yield of oligosaccharides was significantly reduced. Same as or the extraction temperature, it was found that the extraction temperature at 60 °C yielded less of the extract at 30 °C for all treatments. Due to the protein consisting of durian seed starch, the effect of heat-denature at 60 °C, thus, the yield of the extract was decreased. According to Kim *et al.* [36], that study about the extraction of oligosaccharides from defatted soybean starch. When the extraction temperature was 65 °C, the yield of oligosaccharide extract decreased.

The result of the sugar content of crude polysaccharide extract from DSF showed in **Table 2**. In general, the analysis for the determination of free sugar in a sample indicated the total reducing sugar content. Furthermore, the extraction yield and the quantity of non-reducing sugar expressed as oligosaccharides and referred to as prebiotics, were used to determine the extraction efficiency [37]. Non-reducing sugars were calculated as the difference between total sugar and reducing sugars [25]. From the experimental results, the optimal condition for extracting crude polysaccharide from DSF is ethanol 50 % at solvent ratios 1:8 at a temperature of 30 °C for 90 min, gave a maximum extraction yield of 5.28 % and

the maximum amount of non-reducing sugar content at 392.19 ± 10.60 mg mL^{-1} which extraction temperature at 60 °C gave the high non-reducing sugar content at 179.03 ± 4.67 mg mL^{-1}. The high temperature might cause the denaturation of soluble proteins, which thereafter entrapped soluble sugars and impaired the extraction [36]. However, the amount of non-reducing sugar differs depending on the type of raw material, the process of starch preparation before extraction, and the extraction conditions [16].

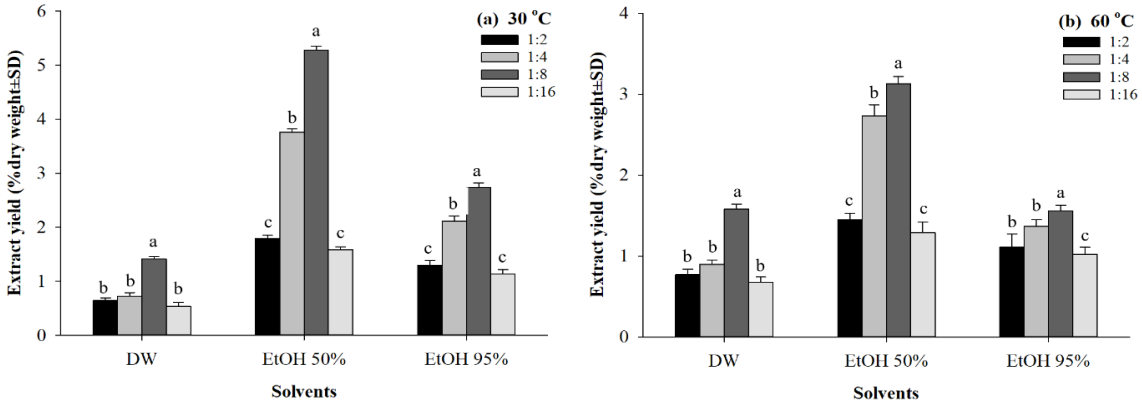

Figure 3 The percentage yield of the crude polysaccharide extract from DSF at solvent ratios 1:2, 1:4, 1:8, and 1:16 using distilled water (DW), ethanol 50 %, and ethanol 95 % at 30 °C for 90 min (a). The percentage yield of the crude polysaccharide extract from DSF at solvent ratios 1:2, 1:4, 1:8, and 1:16 using distilled water (DW), ethanol 50 %, and ethanol 95 % at 60 °C for 90 min (b).

*Different letters mean a significantly different ($p < 0.05$)

***In vitro* digestion studies**

The preliminary evaluation on the prebiotic properties possessed by the extracts. The crude polysaccharide extracts from DSF were tested to resist acidic and enzymatic conditions in the upper digestive system to determine the amounts of their indigestible polysaccharides that can resist digestive processes until reaching the colon and stimulating the bacterial growth to improve host health [38]. This study compared the *in vitro* digestions of crude polysaccharide extracts to inulin as a standard commercial prebiotic (positive control). **Table 3** shows the percentage levels of the extract and inulin hydrolysis after *in vitro* digestion. In the mouth, human salivary α-amylase was utilized for digesting. The percentage of hydrolysis was used to evaluate the capability of the crude extract and inulin. After enzymatic digestion, the extract and inulin exhibited a hydrolysis rate of 2.17 and 0.89 %, respectively. These findings demonstrated that the extract was insensitive to enzymatic digestion and could access the stomach and small intestine with minimum losses of content. Similar to Wong *et al.* [7], the study of enzymatic digestion by human salivary α-amylase to hydrolysis of Artocarpus integer's Seed extract and inulin were 0.16 and 0.09 %, respectively. The crude polysaccharide extracts were continued to hydrolyze with artificial human gastric juice. The percentage levels of the extract and inulin hydrolysis were 3.46 and 1.71 %, respectively. Similar findings were found for pitaya (dragon fruit) flesh, in which the oligosaccharide digestion rate was 2.43 % in gastric juice [39]. After that, the extract was continued to be hydrolyzed with porcine pancreatic α-amylase artificial enzymatic in the small intestine. The percentage levels of the extract and inulin hydrolysis were 9.05 and 8.94 %, respectively. Eq. (4) was used to determine the total percentage of hydrolysis. The total percentage of the extract and inulin hydrolysis after *in vitro* digestion was 14.68 and 11.54 %, respectively. Due to the polysaccharides (galactose, glucose, arabinose, and xylose) present in the crude polysaccharide extracts of DSF, the percentage of hydrolysis of inulin was observed to be lower. However, when the extract was consumed, it was estimated that about 85 % of the compounds reached the colon. Most of the prebiotic substances isolated from DSF can reach the colon without being severely degraded by the acidic and enzymatic environments of the human digestive tract, as demonstrated by these experimental data. The presence of β-glycosidic linkages in the crude polysaccharide extracts may contribute for this reduced digestion. According to Azmi *et al.* [40], these bonds could lead to a non-digestibility of more than 99 %. Due to their resistance to acidic and enzymatic digestion, the results indicated that crude polysaccharide extracts from DSF have the potential to function as prebiotics.

Table 2 The sugar content of the crude polysaccharide extract from DSF at the ratio of durian seed flour to solvent at 1:2, 1:4, 1:8, and 1:16 using distilled water, 50 and 95 % ethanol at 30 and 60 °C for 90 min.

Treatment		Sugar content (mg mL^{-1})					
Solvent	**ratio**	**Total sugar**		**Reducing sugar**		**Non-reducing sugar**	
		30 °C	**60 °C**	**30 °C**	**60 °C**	**30 °C**	**60 °C**
DW	1:2	127.83 ± 10.33^{f}	134.76 ± 6.67^{d}	70.62 ± 4.28^{e}	60.12 ± 4.90^{e}	57.54 ± 7.60^{g}	74.64 ± 10.43^{d}
	1:4	142.14 ± 3.89^{f}	148.23 ± 9.92^{d}	78.47 ± 8.12^{e}	68.07 ± 8.03^{e}	63.67 ± 5.32^{g}	80.16 ± 5.09^{c}
	1:8	318.48 ± 12.43^{cd}	161.48 ± 8.18^{c}	115.16 ± 3.57^{c}	65.36 ± 9.34^{e}	203.32 ± 7.61^{d}	96.12 ± 5.91^{c}
	1:16	92.38 ± 3.21^{g}	79.08 ± 2.16^{e}	62.75 ± 3.19^{f}	50.67 ± 4.21^{f}	31.63 ± 4.21^{h}	28.41 ± 3.07^{e}
EtOH 50 %	1:2	340.09 ± 3.98^{c}	154.91 ± 7.93^{c}	102.14 ± 5.68^{d}	71.42 ± 6.98^{d}	237.95 ± 5.97^{c}	83.49 ± 8.43^{c}
	1:4	402.13 ± 12.32^{b}	321.74 ± 8.02^{b}	113.47 ± 10.32^{c}	152.84 ±5.92^{b}	288.66 ± 8.03^{b}	168.90 ± 4.39^{b}
	1:8	527.67 ± 12.92^{a}	348.16 ± 7.92^{a}	135.48 ± 9.67^{a}	169.13 ± 6.34^{a}	392.19 ± 10.60^{a}	179.03 ± 4.67^{a}
	1:16	208.29 ± 5.2^{e}	165.47 ± 3.44^{c}	119.32 ± 2.97^{c}	89.21 ± 4.37^{c}	88.97 ± 4.36^{f}	76.26 ± 3.55^{d}
EtOH 95 %	1:2	264.28 ± 5.32^{d}	138.40 ± 6.32^{d}	109.84 ± 6.43^{d}	63.13 ± 6.91^{e}	154.44 ± 8.02^{ef}	75.27 ± 5.02^{d}
	1:4	296.94 ± 7.2^{d}	140.83 ± 4.95^{d}	116.78 ± 9.03^{c}	72.06 ± 8.15^{d}	180.16 ± 6.43^{e}	68.77 ± 8.38^{d}
	1:8	287.16 ± 9.32^{d}	152.36 ± 5.09^{c}	122.80 ± 10.39^{b}	70.94 ± 4.98^{d}	164.36 ± 11.08^{e}	81.42 ± 9.38^{c}
	1:16	187.34 ± 3.06^{e}	138.09 ± 3.86^{d}	106.51 ± 3.18^{d}	74.97 ± 4.76^{d}	80.83 ± 2.97^{f}	63.12 ± 3.65^{d}

All results are shown as the mean ± standard deviation of 3 independent measurements. Results in the same column with different superscripts differ significantly ($p < 0.05$)

Table 3 Percentage hydrolysis of extract compared with inulin after *in vitro* digestion.

***in vitro* digestion**	**% Hydrolysis**	
	Crude extract	**Inulin**
Mouth	2.17 ± 1.76^{a}	0.89 ± 2.81^{a}
Stomach	3.46 ± 2.23^{ab}	1.71 ± 3.17^{b}
Small intestine	9.05 ± 2.87^{c}	8.94 ± 1.98^{c}
Total hydrolysis	14.68 ± 3.09	11.54 ± 2.86
Estimated to reach the colon	85.32 ± 3.82	88.46 ± 2.41

All results are shown as the mean ± standard deviation of 3 independent measurements. Results in the same column with different superscripts differ significantly ($p < 0.05$)

Effect of probiotic growth

The crude polysaccharide extract after *in vitro* digestion studies and the reference prebiotic (inulin) were used as a carbon source for the growth of 3 probiotic strains: *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus rhamnosus* TBRC 374 and *Lactobacillus casei* BCC 13300. As shown in **Figure 4**, the crude polysaccharide extract has enhanced activity values higher than inulin with 3 probiotic strains due to carbohydrate content in the extract possibly acting as a different carbon source required by probiotic bacteria. Hence the enhanced activity value increases when the extract concentration increases. However, the values were different in the strains of probiotics tested. *Lactobacillus rhamnosus TBRC* 374 at 5.0 % extract concentration had the highest enhanced activity at 582.67 %. *Lactobacillus casei BCC* 13300 and *Lactobacillus acidophilus* ATCC 4356 with enhanced activity were 543.78 and 288.34 %, respectively. Because polysaccharides can encourage the growth of lactic acid bacteria, this consequence occurs. However, the various non-reducing sugar types, each of which has various carbon sources, impact the lactic acid bacteria. Different bacterial species have different rates of growth. By sugar metabolism, lactic bacteria can be divided into 3 groups: homofermentative, required heterofermentative, and facultative heterofermentative [41]. The Embden Mayerhof Parnas (EMP) pathway can only break down 6-carbon

sugars like arabinose, xylose, and others but cannot break down 5-carbon sugars like glucose, fructose sugar, galactose, and hexose sugar. The heterofermentative bacteria *Lactobacillus rhamnosus* and *Lactobacillus casei* can use both 5-carbon and 6-carbon sugars, while the homofermentative bacteria *Lactobacillus acidophilus* can use only 6-carbon sugars. These findings demonstrate the excellent prebiotic properties of crude extracts of DSF, which can promote the growth of probiotic bacteria. Additionally similar with the discoveries that jackfruit seed oligosaccharides [42]. The primary property of prebiotics is their capacity to promote the growth of probiotics or beneficial microorganisms that comprise the human colonic microbiome, according to Gibson *et al.* [37].

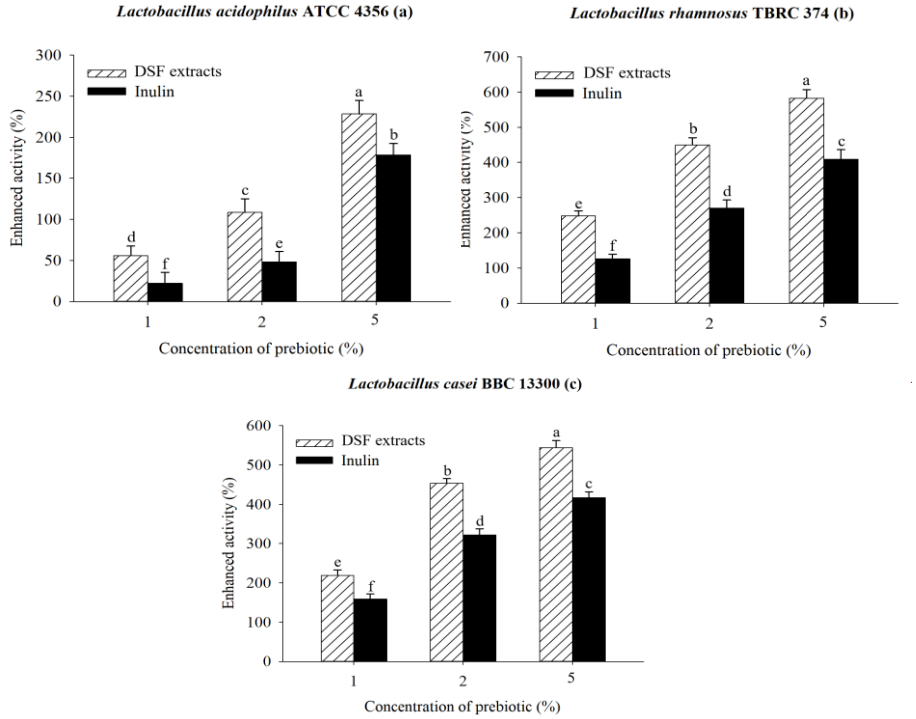

Figure 4 Enhanced activity of probiotic (a) *Lactobacillus acidophilus* ATCC 4356, (b) *Lactobacillus rhamnosus* TBRC 374, and (c) *Lactobacillus casei* BCC 13300 when cultured in MRS medium with samples of the extract from durian seed flour at concentrations of 1.0, 2.0 and 5.0 % (w/v) compared to commercial prebiotics (inulin).
*Different letters mean a significantly different ($p < 0.05$)

Conclusions

This study demonstrated that durian seed flour contains a substantial quantity of potentially prebiotic substances. The optimal condition for extracting crude polysaccharide from DSF is ethanol 50 % at solvent ratios 1:8 at a temperature of 30 °C for 90 min, gave a maximum extraction yield of 5.28 % and the maximum amount of non-reducing sugar content at 392.19 ± 10.60 mg mL^{-1}. The crude polysaccharide extract resisted gastric juice and enzyme digestion. In the *in vitro* digestion investigation, it was estimated that approximately 85 % of them reached the colon. Moreover, the crude polysaccharide extract stimulated the growth of probiotics, including *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus rhamnosus* TBRC 374, and *Lactobacillus casei* BCC 13300, more effectively than the commercial prebiotic inulin. Thus, both durian seed flour and crude polysaccharide extract have the ability to function as a prebiotic and can be utilized as a component in the development of functional foods.

Acknowledgements

The authors are grateful for the financial support provided by the Kasetsart University Thailand Research and Development Institute (KURDI), FF(KU)34.65. Many thanks to the Department of Applied Microbiology, Institute of Food Research and Product Development, Kasetsart University Thailand, and the Division of Biological Science, Faculty of Science, Prince of Songkla University Thailand, for their technical support.

References

[1] CE West, H Renz, MC Jenmalm AL Kozyrskyj, KJ Allen, P Vuillermin and SL Prescott. The gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies. *J. Allergy Clin. Immunol.* 2015; **135**, 3-13.

[2] International Scientific Association for Probiotics and Prebiotics, Available at: https://isappscience.org/for-consumers/learn/prebiotics, accessed June 2022.

[3] CJ Ziemer and GR Gibson. An overview of probiotics, prebiotics and synbiotics in the functional food concept: Perspectives and future strategies. *Int. Dairy J.* 1998; **8**, 473-9.

[4] P Gulewicz, D Ciesiolka and J Frias. Simple method of isolation and purification of alpha - galactosides from legumes. *J. Agr. Food Chem.* 2000; **48**, 3120-3.

[5] M Vrese and J Schrezenmeir. Probiotics, prebiotics, and syn biotics, in food biotechnology. *Adv. Biochem. Eng. Biotechnol.* 2008; **111**, 1-66.

[6] T Varzakas, P Kandylis, D Dimitrellou, C Salamoura, G Zakynthinos and C Proestos. Innovative and fortified food: Probiotics, prebiotics, GMOs, and superfood. *Preparation Process. Relig. Cult. Foods* 2018; **2018**, 67-129.

[7] W Joel-Ching-Jue, H Siew-Ling and K Chen-Chung. Isolation of prebiotics from *artocarpus integer's* seed. *Int. J. Food Sci.* 2021; **2021**, 9940078.

[8] G Mandalari, C Nueno-Palop, G Bisignano, MSJ Wicham and A Narbad. Potential prebiotic properties of almond (*Amygdalus communis L.*) seeds. *Appl. Environ. Microbiol.* 2008; **74**, 4264-70.

[9] E Menne and N Guggenbuhl. Fn-type chicoryinulin hydrolysate has a prebiotic effect in humans. *J. Nutr.* 2000; **130**, 1197-9.

[10] FAO, Available at: http://www.fao.org, accessed July 2022.

[11] X Fang, Y Li a, YL Kua, ZL Chew, S Gan, KW Tan, TZE Lee, WK Cheng and HLN Lau. Insights on the potential of natural deep eutectic solvents (NADES) to fine-tune durian seed gum for use as edible food coating. *Food Hydrocolloids* 2022; **132**, 107861.

[12] T Tongdang. Some properties of starch extracted from three Thai aromatic fruit seeds. *Starch* 2008; **60**, 199-207.

[13] D Malini, I Arief and H Nuraini. Utilization of durian seed flour as filler ingredient of meatball. *Media Peternakan* 2016; **39**, 161-7.

[14] UN Hakim, D Rosyidi and AS Widati. The effect of arrowroot flour (*Maranta arrundinaceae)* on physical and sensoric quality of rabbit nugget. *Jurnal Ilmu Teknologi Hasil Ternak* 2013; **8**, 9-22.

[15] BT Amid and H Mirhosseini. Optimisation of aqueous extraction of gum from durian (*Durio zibethinus*) seed: A potential, low-cost source of hydrocolloid. *Food Chem.* 2012; **132**, 1258-68.

[16] BT Amid, H Mirhosseini and S Kostadinovic. Chemical composition and molecular structure of polysaccharide-protein biopolymer from *Durio zibethinus* seed: Extraction and purification process. *Chem. Cent. J.* 2012; **6**, 117-34.

[17] MD Torres, R Moreira, F Chenlo, MH Morel and C Barron. Physicochemical and structural properties of starch isolated from fresh and dried chestnuts and chestnut flour. *Food Tech. Biotechnol.* 2014; **52**, 135-9.

[18] RA Anderson, HF Conway, VFP feifer and EL Griffin. Gelatinisation of corn grits by roll and extrusion cooking. *Cereal Sci. Today* 1969; **14**, 4-12.

[19] AOAC 2019, Available at: https://www.aoac.org, accessed May 2022.

[20] MC Coimbra and N Jorge. Proximate composition of guariroba (*Syagrus oleracea*), jeriva (*Syagrus romanzoffiana*) and macauba (*Acrocomia aculeate*) palm fruits. *Food Res. Int.* 2011; **44**, 2139-42.

[21] N Singh, J Singh, L Kaur, NS Sodhi and BS Gill. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chem.* 2003; **81**, 219-31.

[22] M Dobois, KA Gilles, JK Hamilton, PA Rebers and F Smith. Colorimetric method of determination of sugars and related substances. *Anal. Chem.* 1956; **28**, 350-6.

[23] GC Miller. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 1959; **31**, 426-8.

[24] JA Robertson, P Ryden, RL Botham, S Reading, G Gibson and SG Ring. Structural properties of diet-derived polysaccharides and their influence on butyrate production during fermentation. *LWT Food Sci. Tech.* 2001; **34**, 567-73.

[25] ABJ Lakho1, AH Soomro and HHM Hammad. Effects of pectin on the reducing and non-reducing sugar and total sugar percentage of date jam. *J. Biol. Agr. Healthc.* 2017; **7**, 84-7.

[26] C Fassler, E Arrigoni, K Venema, V Hafner, F Brouns and R Amadò. Digestibility of resistant starch containing prepa-rations using two *in vitro* models. *Eur. J. Nutr.* 2006; **45**, 445-53.

[27] R Desaesamoh. 2014, Extraction and purification of oligosaccharide from dragon fruit's flesh and evaluation of prebiotic properties. Master Thesis. Prince of Songkla University, Songkla, Thailand.
[28] K Phothichitto, S Nitisinprasert and S Keawsompong. Isolation, screening and identification of mannanase producing microorganisms. *Kasetsart J.* 2006; **40**, 26-38.
[29] AM Amin, AS Ahmad, YY Yin, N Yahya and N Ibrahim. Extraction, purification and characterization of durian (*Durio zibethinus*) seed gum. *Food Hydrocolloids* 2007; **21**, 273-9.
[30] ND Permatasari, JE Witoyo, M Masruri, SS Yuwono and SB Widjanarko. Nutritional and structural properties of durian seed (*Durio zibenthinus* Murr.) flour originated from West Kalimantan, Indonesia. *IOP Conf. Ser. Earth Environ. Sci.* 2022; **1012**, 012038.
[31] S Baraheng and T Karrila. Chemical and functional properties of durian (Durio zibethinus Murr.) seed flour and starch. *Food Biosci.* 2019; **30**, 100412.
[32] Y Aboubakar, N Njintang, J Scher and CMF Mbofung. Physicochemical, thermal properties and microstructure of six varieties of taro (*Colocasia esculenta L. Schott*) flours and starches. *J. Food Eng.* 2008; **86**, 294-305.
[33] P Wiriyawattana, S Suwonsichon and T Suwonsichon. Effects of drum drying on physical and antioxidant properties of riceberry flour. *Agr. Nat. Res.* 2018; **52**, 445-50.
[34] P Leemud, S Karrila, T Kaewmanee and T Karrila. Functional and physicochemical properties of Durian seed flour blended with cassava starch. *J. Food Meas. Char.* 2020; **14**, 388-400.
[35] X Xiaoli, Y Liyi, H Shuang, L Wei, S Yi, M Hao, Z Jusong and Z Xiaoxiong. Determination of oligosaccharide content in 19 cultivars of chickpea seeds (*Cicerarietinum L.*) by HPLC. *Food Chem.* 2008; **11**, 215-9.
[36] S Kim, W Kim and IK Hwang. Optimization of the extraction and purification of oligosaccharides from defatted soybean meal. *Int. J. Food Sci. Tech.* 2003; **38**, 337-42.
[37] CY Lee, RS Shallenberger and MT Vittum. Free sugar in fruits and vegetable. *Food Sci. Tech.* 1970; **1**, 1-12.
[38] GR Gibson, HM Probert, JAEV Loo, RA Rastall and MB Roberfroid. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutr. Res. Rev.* 2004; **17**, 259-75.
[39] S Wichienchot, M Jatupornpipat and RA Rastall. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chem.* 2010; **120**, 850-7.
[40] AF Azmi, S Mustafa, DM Hashim and YA Manap. Prebiotic activity of polysaccharides extracted from gigantochloa levis (*Buluh beting*) shoots. *Molecules* 2012; **17**, 1635-51.
[41] O Kandler and N Weiss. *Regular, non-sporing Gram-positive rods*. *In*: HA Sneath, NS Mair, ME Sharpe and JG Holt (Eds.). Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, Maryland, 1986.
[42] P Thammarutwasik, T Hongpattarakere, S Chantachum, K Kijiroongrojana, A Itharat, W Reanmongkol, S Tewtrakul and B Ooraikul. Prebiotics: A review. *Songklanakarin J. Sci. Tech.* 2009; **31**, 401-8.