Evaluation and characterisation of candidate prebiotics extracted from coconut husk by ultrasound-assisted extraction technique

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Abstract. Oligosaccharides are carbohydrates containing between three to ten sugar moieties. Certain oligosaccharides such as inulin and fructo-oligosaccharides are known as prebiotics that promote the growth of beneficial bacteria in the human gastrointestinal tract. This study began by comparing the efficiency of two different solvents (distilled water and 10% w/v sodium hydroxide) in extracting oligosaccharides from the coconut husk by ultrasound-assisted extraction (UAE). Following that, the coconut husk extract (CHE) extract was subjected to a series of prebiotic evaluation tests. The findings indicated that a significantly high extraction yield (40.51 ± 6.00%) could be achieved with 10% w/v NaOH treatment. The in vitro enzymatic digestion study found that there was 43.70 ± 0.15% of hydrolysis at pH 8 after five hours of incubation. For the in vitro gastric juice digestion, 29.21 ± 0.71% of hydrolysis was recorded at pH 1 after four hours of incubation. The extract was able to stimulate the growth of selected beneficial bacterial strains. FTIR and NMR analysis of the CHE revealed that the extract has a similar structure to the well-known prebiotic inulin.

1. Introduction

Coconut (Cocos nucifera) is cultivated in 92 countries, with Indonesia producing the most [1]. Malaysia is one of the world’s top ten coconut-producing countries. After oil palm, rubber, and rice, coconut is Malaysia’s fourth most important industrial crop [2]. Coconut husks, shells, and fronds are the most common agricultural waste generated during the harvesting and processing of coconuts in the industry [3]. Instead of being disposed of directly, these agro-wastes can be converted into value-added products. Coconut husks contain 38% hemicellulose, 28% cellulose, and 32.8% lignin [4]. Due to the high concentration of hemicellulose in coconut husk, it could be a source of oligosaccharides with prebiotic properties.

Prebiotics were originally defined as “non-digestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host health” [5]. Numerous dietary oligosaccharides and polysaccharides (including dietary fibre) have been claimed to possess prebiotic properties. Oligosaccharides are carbohydrates which made up of 3 to 20 sugar monomer units.

Gibson et al. (2004) [6] state that a food ingredient must meet three criteria to be classified as a prebiotic: To begin, a prebiotic must be resistant to digestive processes such as gastric acidity,
mammalian enzyme hydrolysis, and gastrointestinal absorption. Second, it must be fermented by the microbiota in the intestine. Thirdly, it must stimulate the growth and activity of beneficial intestinal microbiota in a selective manner.

Extraction of bioactive compounds from natural resources using conventional methods is time consuming and has a low extraction efficiency. Innovative technologies, such as ultrasound-assisted extraction (UAE), have been investigated to overcome these disadvantages. UAE has been used to extract numerous bioactive compounds from a variety of animal, plant, and food matrices [7]. UAE is highly regarded for its straightforward protocols, low investment costs, and high efficiency. UAE's primary advantages are a reduction in extraction time and energy consumption. Reduced operating temperature and processing time are particularly advantageous for heat-labile compound extraction. Apart from expediting extraction, UAE maintains molecular and structural properties [8].

The purpose of this study was to determine the feasibility of extracting prebiotic oligosaccharides from coconut husk using UAE. The UAE was conducted by comparing the effects of two different solvents on the oligosaccharide extract’s chemical properties in terms of total carbohydrate (TC), total reducing sugar (TRS), and degree of polymerisation (DP). Prebiotic potency tests were performed on the resulting oligosaccharide extract from coconut husk. Following that, the molecular structure of coconut husk extract (CHE) was compared to that of a well-characterized prebiotic (inulin) using Fourier Transform Infrared (FTIR) and Nuclear Molecular Resonance (NMR) spectroscopy.

2. Materials and Methods

2.1. Sample Preparation
The sample was prepared based on the method proposed by Wichienchot et al. (2011) [9] with modification. Coconut husk was cleaned thoroughly under running tap water after collection from the local market. The coconut husk was dried in the oven until constant weight and then grinded into fine powder. The powder was sieved to pass through 250 µm mesh sieve and stored in a clean container at ambient temperature until further analysis.

2.2. Preliminary Study: Selection of Extraction Solvents
The preliminary study was conducted based on the method proposed by Alcántara et al. (2020) [10] with modification. There were two solvents used in the preliminary UAE process: (1) distilled water (dH₂O), and (2) 10% w/v sodium hydroxide (NaOH) solution. Approximately two grams of coconut husk powder was mixed with 200 mL of distilled water (solid-to-liquid ratio 1:100). The mixture was then subjected to UAE at two different powers (25 W and 250 W) and time intervals of 5 min and 45 min, respectively.

After UAE, the mixture was centrifuged at 4500 rpm, 4 °C for 10 min. The resulting supernatant was subjected to overnight precipitation with three volumes of cold ethanol (EtOH). After that, the solution was centrifuged at 4500 rpm, 4 °C for 10 min to separate the precipitate. The precipitate that contained the extracted oligosaccharides was collected, dried at 60 °C until constant weight. The total carbohydrates (TC) content, total reducing sugar (TRS) content and degree of polymerisation (DP) of the extracted oligosaccharide was then determined (Section 2.5). All the extraction was conducted at temperature of 30 °C in replicates. The UAE was also performed by replacing the distilled water with 10% w/v NaOH solution.

2.3. Determination of Total Carbohydrates Content
Phenol-sulfuric acid assay was used to determine the total concentration of carbohydrates in the extract by using glucose as standard solution [11]. One millilitre of extract was mixed with 5% phenol solution (1 mL) and concentrated sulphuric acid (5 mL). After vortex, the mixture was incubated in a 30°C water bath for 30 min. After that, the absorbance of the mixture was measured at 490 nm.
2.4. Determination of Total Reducing Sugar (DNSA Analysis)
The concentration of reducing sugar was determined by 3, 5-dinitrosalicylic acid (DNSA) method [12]. One millilitre of the extract was mixed with 1 mL of DNS reagent followed by two drops of 10% sodium hydroxide and boiled for 15 min. The mixture was cooled under running tap water. Next, 10 mL of distilled water was added and vortexed. The mixture was left for 20 min and the absorbance was measured at 540 nm.

2.5. Degree of Polymerisation
The degree of polymerisation of the oligosaccharides was calculated using the equation (1).

\[
Degree\ of\ polymerisation = \frac{\text{Total Carbohydrates}}{\text{Total Reducing Sugar}}
\]  

(1)

2.6. In vitro Fermentation Study with CHE as Sole Carbon Source
A single colony of the bacterial cultures (Lactobacillus acidophilus DSM 20079, Lactobacillus casei DSM 20011 and Bifidobacterium bifidum DSM 20239, and Escherichia coli DSM 1103) from respective agar plate was transferred into MRS broth or tryptic soy (TS) broth accordingly. The probiotic bacteria were pre-cultured in MRS broth at 37 ± 1 °C for 48 to 72 hours while the E. coli in TS broth were cultured for 24 hours. In order to investigate the effect of CHE on the growth of the bacteria, both MRS and TS fermentation media with a series of concentrations of CHE (0.5% w/v to 3.0% w/v) were prepared to replace the glucose.

Next, the medium was inoculated with 1 mL of bacterial culture (approximately 10⁴ CFU/mL) and incubated at 37 ± 1 °C for up to 72 hours. Inoculated medium without glucose served as negative control while medium with inulin served as positive control [13]. All incubation, fermentation and enumeration of those probiotic bacteria were performed in an anaerobic system (Oxoid AnaeroJar™ 2.5L, UK), while that of E. coli was performed in an aerobic system. The viable count of the cultures (CFU/mL) was determined at predetermined time intervals. The result was expressed as log₁₀ CFU/mL.

The short chain fatty acid (SCFA) namely, lactate, acetate, propionate and butyrate in the fermented culture broth were identified and quantified using High Performance Liquid Chromatography (HPLC) method with minor modifications [14].

2.7. In vitro Enzymatic Digestion
In this analysis, human salivary α-amylase suspension was used to mimic the upper gastrointestinal digestion. The in vitro enzymatic digestion was carried out according to the method by Fässler et al. (2006) [15].

First, sodium phosphate buffer solution with concentration of 20 mM was prepared. Approximately 3.12 g sodium hydrogen orthophosphate (NaH₂PO₄) and 0.392 g sodium chloride (NaCl) was dissolved with approximately 900 mL distilled water. After that, the pH of the buffer was adjusted to pH 7.0 by using 0.1 N sodium hydroxide (NaOH) solution. The buffer was transferred to a 1 L volumetric flask and made up to volume with distilled water. Human salivary α-amylase was then dissolved in the 20 mM sodium phosphate buffer.

In a clean test tube, 5 mL of the enzyme solution and 5 mL of the extract solution were mixed. The test tube was then incubated in the water bath at 37 ± 1 °C for 5 hours. The mixture was withdrawn in fixed time interval: 0, 1, 2, 3, 4 and 5 hours of post incubation. The total carbohydrates content was measured using phenol-sulphuric acid method whereas the initial and final reducing sugar contents were measured using DNSA assay.

The percentage hydrolysis of the extract was calculated based on the reducing sugar liberated and the total carbohydrates content by using equation (2) [16].
where reducing sugar liberated = final reducing sugar content – initial reducing sugar content

2.8. In vitro Artificial Gastric Juice Digestion

The gastric juice digestion analysis was carried out based on the method proposed by Wichienchot, Jatupornpipat and Rastall (2010) [17]. In this analysis, inulin was used as positive control. The samples and inulin were incubated with the artificial gastric juice for predetermined duration.

There were two steps involved for the preparation of artificial gastric juice. First, a phosphate buffer solution was prepared. Then, 5 M HCl was used to adjust the pH of the buffer solution to 1, 2, 3 and 4. The resulting solutions were known as artificial gastric juice.

In the meantime, 1% (w/v) of CHE was prepared. The total carbohydrates content and initial reducing sugar content were measured using phenol sulphuric acid analysis and DNSA assay respectively.

Five millilitres of 1% (w/v) CHE were mixed with 5 mL of HCl buffer solution and incubated in a water bath at 37 ± 1 °C for 5 hours. The mixture solutions were withdrawn for analysis at the time interval of 0, 1, 2, 3, 4 and 5 hours post incubation [17]. The final reducing sugar content were measured using DNSA assay. Percent of hydrolysis was calculated by using equation (2) [16].

2.9. Fourier Transform Infrared (FTIR) Spectroscopy

The structure of extracted oligosaccharides from coconut husk was characterised with Fourier transform infrared spectroscopy (FTIR). The analysis was conducted using Thermo Nicolet iS10 FTIR spectrometer with the resolution of 0.4 cm⁻¹ and the spectral range is 7800 cm and 350 cm. Dried CHE were ground into fine powder prior analysis. Spectral scanning was taken between the wavenumber of 4000 cm⁻¹ and 650 cm⁻¹. Inulin was used as a standard.

2.10. Nuclear Magnetic Resonance (NMR) Spectroscopy

The structure of oligosaccharides extracted from coconut husk was further identified by using the ¹H and ¹³C NMR spectroscopy [18]. The NMR spectra were recorded using a Varian NMR spectrometer system operating at frequency of 500 MHz. The oligosaccharide was dissolved in deuterated water (D₂O) for both ¹H and ¹³C NMR. Inulin was used as a standard.

3. Results and Discussion

3.1. Preliminary Studies: Selection of Extraction Solvents

As shown in table 1, using NaOH as the extracting solvent results in a higher percentage of recovery than using distilled water. The preliminary study found that extracting oligosaccharides from coconut husk with NaOH solution was more effective than extracting with distilled water.

For total carbohydrates, water extraction at 250 W for 45 min produced 0.72 mg/mL of total carbohydrates. The lowest total carbohydrates content was obtained through water extraction at ultrasonic power of 250 W for 5 min. This could be attributed to the shorter extraction duration and hence less surface contact between the solvents and sample [19, 20]. In contrast, NaOH extraction produced a significantly higher amount of total carbohydrates.

There was no significant difference in the total reducing sugar content of oligosaccharides extracted from coconut husks using distilled water regardless of the extraction conditions. Nonetheless, when NaOH was used for extraction, a longer extraction time resulted in a higher TRS. The prolonged extraction time results in increased cavitation of microbubbles. The formation of bubbles increases the contact surface area and scours the surfaces of the sample [21].
Table 1. Comparison of two solvents used on extraction of oligosaccharide from coconut husk.

| Chemical properties | Type of solvent | Extraction condition |
|---------------------|-----------------|----------------------|
|                     | 5 min, 25 W     | 5 min, 250 W         | 45 min, 25 W    | 45 min, 250 W |
| Total carbohydrates (TC) |                |                      |                |               |
| dH2O                | 0.38 ± 0.35 b, c | 0.06 ± 0.03 b, c     | 0.17 ± 0.06 b, c | 0.72 ± 0.52 b, c |
| NaOH                | 120.29 ± 1.20 b  | 110.51 ± 2.99 b      | 120.07 ± 2.75 b | 108.19 ± 5.59 b |
| Total reducing sugar (TRS) |            |                      |                |               |
| dH2O                | 0.005 ± 0.01 b, c | 0.178 ± 0.23 b, c   | 0.016 ± 0.02 b, c | 0.076 ± 0.11 b, c |
| NaOH                | 22.18 ± 1.80 b, d | 21.62 ± 0.06 b, d | 19.68 ± 1.52 b, d | 48.17 ± 0.20 b, d |
| Degree of polymerisation (DP) | |                    | | |
| dH2O                | 76.0            | 0.34                 | 10.63           | 9.47           |
| NaOH                | 5.42            | 5.11                 | 6.10            | 2.25           |
| % Recovery         | dH2O            | 0.555 ± 0.51 b, c    | 0.066 ± 0.01 b, c | 0.114 ± 0.01 b, c | 0.435 ± 0.22 b, c |
|                    | NaOH            | 40.49 ± 0.24 b, f    | 40.51 ± 6.00 b, f | 40.37 ± 5.13 b, f | 34.95 ± 16.82 b, f |

a All the values (except for degree of polymerisation) are expressed as mean ± SD of replicates for the same group of chemical property.

b Values within the same rows for same type of solvent followed by different small letters are significantly different (p < 0.05).
c Values within the same columns for same chemical properties followed by different capital letters are significantly different (p < 0.05).

As shown in table 1, for NaOH extraction, a longer extraction time (45 min) combined with a 250W ultrasonic power resulted in the highest concentration of TRS. As indicated by the lower level of TRS, the lower ultrasonic power (25 W) may not be strong enough to degrade the carbohydrate derivatives in the sample to a simpler form of sugar.

After the total carbohydrates and total reducing sugar were determined, the degree of polymerisation (DP) was calculated using equation (1). In order to obtain high DP value, high total carbohydrates content and low total reducing sugar content are desirable [22].

3.2. In vitro Fermentation Study

For in vitro fermentation study, coconut husk extract (CHE) was used as the sole carbon source for cultivation of selected beneficial bacterial strain, namely Lactobacillus acidophilus DSM 20079, Lactobacillus casei DSM 20011 and Bifidobacterium bifidum DSM 20239, and one available pathogenic strain in the laboratory, i.e., Escherichia coli DSM 1103. Inulin was used as the positive control in this study. The colonies forming unit for each strain was determined and expressed as log_{10} CFU/mL. In addition, pH of the cultivation broth collected at specific time interval was measured. The broth was then centrifuged prior to total carbohydrates and short chain fatty acid (SCFA) determination. The profile of microbes’ growth, pH changes and the total carbohydrates consumption were determined. The growth rate (CFU per hour of incubation) and the growth yield (CFU per g of substrate) were calculated.

Figure 1 shows the growth rate (a) and growth yield (b) of respective strains in medium containing different concentrations of CHE (0.5% w/v to 3.0% w/v), in comparison with inulin as sole carbon sources. Through the concentration-dependent analysis, the effect of CHE on the probiotic growth can be evaluated. From the results, all the tested bacteria could utilise CHE. The proliferation effect decreased in the order from B. bifidum, followed by E. coli, L. casei and then L. acidophilus in media containing 1.0% w/v of CHE.

3.2.1. L. acidophilus. As shown in the figure 1 (a), the average growth rate of L. acidophilus in media using 2.0% w/v and 3.0% w/v of CHE are significantly higher as compared to the 1.0% w/v inulin. Similar growth rate was observed in the media with the substrate concentrations of 0.5%, 1.0% and 1.5% (w/v) CHE. In contrast, there is no significant difference (p > 0.05) in the growth rate of L. casei, B. bifidum and E. coli cultivated with 1.0% w/v CHE and 1.0% w/v inulin although the growth rate of the three strains in both medium are higher than medium with 0.5%, 1.5%, 2.0% and 3.0% w/v CHE.
Moreover, 1.0% w/v CHE shows mean growth yield of $25.43 \pm 3.58$ log CFU per mg of substrate on L. acidophilus (figure 1 (b)). The medium with 1.0% w/v inulin gives the lowest growth yield of $3.96 \pm 0.91$ log CFU per mg of substrate among the other substrates used.

3.2.2. L. casei. In medium with 1% w/v of respective substrate, the average growth rate of L. casei was higher compared to other substrates while similar growth rate observed when L. casei was grown in medium with 0.5%, 1.5%, 2.0% and 3.0% (w/v) of CHE. In addition, the effect of 1.0% w/v CHE was positive as it stimulates the growth yield of L. casei of $20.17 \pm 0.97$ log CFU per mg of substrate. On the other hand, the cell density of L. casei grown in media with 1.0% w/v inulin was the lowest with growth yield of $5.25 \pm 1.03$ log CFU per mg of substrate.

![Figure 1](image_url)

**Figure 1.** (a) Growth rate and (b) Growth yield of L. acidophilus, L. casei, B. bifidum and E. coli in medium with different concentration of CHE (% w/v). Symbols: (●), 0.5%; (□), 1.0%; (□□), 1.5%; (□□□), 2.0%; (□□□□), 3.0%; (□□□□□) 1.0% inulin. Error bars indicate the mean ± standard deviation of three replicates. The error bars were smaller than the size of the symbols as regards to the data points without error bars.
3.2.3. *B. bifidum*. The mean growth rate of *B. bifidum* is high in medium with 1.0% w/v CHE and the growth rate was similar in medium with 1.0% w/v inulin, with growth yield of 94.10 ± 43.97 log CFU per mg of substrate. The growth yield of *B. bifidum* reduced when higher concentration of CHE (above 1.0% w/v) was used as carbon source in the medium. Overall, CHE possess noticeable proliferation effect of the beneficial microorganism with significant growth stimulation effect on *B. bifidum*, followed by *L. casei* and *L. acidophilus*, in comparison with inulin. Similar findings were reported by Lee *et al.* (2002) [23] who were using chitosan oligosaccharides (COS) as the carbon source on the growth of selected strains. Another study using oligosaccharides from jackfruit seed also have been reported to show larger growth stimulatory effect on lactobacilli sp. and bifidobacterium sp. [24].

3.2.4. *E. coli*. In addition to beneficial microbe strains mentioned above, the growth profile of a pathogenic microbe – *E. coli*, was also conducted. As shown in figure 1 (a), by using 1.5 % w/v CHE, higher growth rate of *E. coli* was achieved with the highest average growth yield of 75.48 ± 6.97 log CFU per mg of substrate. According to Fooks and Gibson (2002) [25], the enteropathogenic bacteria, such as *E. coli*, *C. jejuni* and *S. enteriditis*, were able to utilise the different prebiotic as growth substrates, namely FOS, inulin, inulin:FOS mixture (80:20, w/w), XOS, FOS:XOS mixture (50:50, w/w), lactulose, lactitol, starch and dextran, to support their growth.

Of the four species compared with media containing 1.0% w/v CHE, *B. bifidum* grew the fastest with the lowest pH value of fermentation medium recorded. A reduction in pH value indicates the substrates being metabolized and produced organic acid by the strains [11]. Besides, the differences of the growth pattern might due to the chemical composition and molecular conformation of the coconut husk extract [26].

For example, the sugar content of the oligosaccharides could affect the proliferation effect [27]. The proliferation effect of inulin was more significant than CHE probably because of the higher sugar content. Molecular weight is one of the factors that have an effect on the accessibility of oligosaccharides to probiotic strains [27]. Furthermore, the branch degree of the oligosaccharides might influence the prebiotic activity too. Chain length is likely to be one of the contributory factors, since with long chain and multiple branching, more hydrolysis by the organisms are required for the fermentation [25]. According to Stewart, Timm and Slavin (2008) [28], the branched FOS have stronger prebiotic effect than unbranched FOS. Last but not least, water solubility is another important factor. The oligosaccharides with good water solubility could more easily and completely be utilised by the microorganism [29]. Aside from that, there might be other mechanisms that need to be studied and disclosed in the future.

3.2.5. Time Course of Total Short-chain Fatty Acid (SCFA) Production. Figure 2 shows the time course profile of the total amounts of short chain fatty acid (SCFA) produced by selected strains in medium with different concentration of CHE. The type of SCFA detected are lactic acid, acetic acid, propionic acid and butyric acid. For *L. acidophilus* strain, the total SCFA produced by using 1.0% w/v CHE and 1.0% w/v inulin are 11.16 ± 0.06 g/L and 11.42 ± 0.13 g/L, respectively after 72 h of cultivation. The results indicate that CHE could produce comparable amount of SCFA as inulin when utilised as carbon source during the fermentation process.
For \textit{L. casei} strain, 13.50 ± 0.03 g/L of total SCFA was produced when using 1.0% w/v CHE as carbon sources for 72 h of fermentation. In contrast, significantly low level of total SCFA was produced (3.81 ± 0.001 g/L) in medium with 0.5% w/v CHE. When \textit{B. bifidum} was cultivated in 1.0% w/v CHE, there was 8.97 ± 0.01 g/L of total SCFA produced after 24 h of fermentation. There was a notably decrease of the total SCFA observed after 24 h in medium with 1.0% w/v inulin.

The results of present study indicated that the amount of 1.0% w/v CHE is sufficient for the cultivation of \textit{L. acidophilus}, \textit{L. casei} and \textit{B. bifidum} strains for SCFA production. The strains utilise the carbon sources to support the growth throughout the cultivation period. The fermentation of the carbon substrate resulted the production of SCFA as the product throughout the fermentation process [30]. There is no SCFA production observed after 24 h of fermentation with 0.5% and 1.0% w/v of CHE on \textit{E. coli} (figure 2 (d)). This might reflect the substrate used do not support the growth of \textit{E. coli} for more than 24 hours. Without the growth of the microbes, no production of total SCFA could be detected. Most of the SCFA produced was lactic acid followed by acetic acid. The anaerobic growth condition was reported to force the microbes in utilising the anaerobic pathway to produce the lactic acid [31].

3.3. \textit{In vitro Enzymatic Digestion}

The enzyme used in this study was human salivary \(\alpha\)-amylase. The ability of coconut husk extract (CHE) and inulin to resist enzymatic digestion was determined based on the percentage of hydrolysis (equation (2)). Figure 3 (a) and (b) shows the percentage of hydrolysis of coconut husk extract (CHE) and inulin after enzymatic digestion by salivary \(\alpha\)-amylase at pH 6, pH 7 and pH 8.
Figure 3. Percentage of hydrolysis (a) coconut husk extract (CHE) and (b) inulin after enzymatic digestion at various pH, incubated at 37 °C for five hours. Symbols: (■), pH 6; (▲), pH 7; (●), pH 8. Error bars indicate the mean ± standard deviation of three replicates. The error bars were smaller than the size of the symbols as regards to the data points without error bars.

The hydrolysis of coconut husk extract increased significantly within first few hours. The maximum percentage of hydrolysis of coconut husk extract with human salivary α-amylase at pH 6, pH 7 and pH 8 was 37.56 ± 0.04%, 40.37 ± 0.04% and 43.70 ± 0.15%, respectively after 5 h of incubation. Meanwhile, the inulin hydrolysis is mainly occurred at pH 6, 7 and 8 with 32.36 ± 0.03%,
38.73 ± 0.03% and 42.49 ± 0.03% recorded, respectively. Based on the findings, alkaline pH conditions gave a significantly higher degree of hydrolysis, indicating the optimal pH for α-amylase activity was in the range of 5.5 to 7.5 [32]. Wang et al. (2015) [27] also reported that the digestibility of rapeseed polysaccharides at different pH values were in the order of pH 7 > pH 8 > pH 6 > pH 5.

By comparison of the coconut husk extract and inulin, the percentage of hydrolysis of coconut husk extract has significantly differences (p < 0.05). From the present study, approximately 30 to 40% of coconut husk extract was expected to reach the colon and utilised by the probiotic bacteria.

3.4. In vitro Artificial Gastric Juice Digestion

The coconut husk extract (CHE) was subjected to in vitro artificial gastric juice digestion. After five hours of incubation, the content of fermentable sugar in the solution was determined. Throughout the gastric juice digestion test, the reducing sugar content increased after digestion due to hydrolysis of the glycosidic bonds of CHE that would increase the amount of reducing sugars. The increase of the total reducing sugar then further contributed to the increase of the percentage of hydrolysis.

Figure 4 (a) and (b) show the percentage of hydrolysis of coconut husk extract and inulin after gastric juice digestion at pH 1 to 4 for five hours, respectively. The percentage of hydrolysis was calculated using equation (2). The solution was collected at every hour interval immediately after the incubation with artificial gastric juice. The percentage of hydrolysis of coconut husk extract (CHE) increased when highly acidic artificial gastric juice was used. The degree of hydrolysis at pH of 1, 2, 3 and 4 are 24.00 ± 0.70%, 14.90 ± 0.65%, 16.79 ± 0.11% and 9.98 ± 0.52%, respectively after two hours of incubation.

The gastric juice with pH 2 to pH 4 was generally released within 2 h after the consumption of food [17]. At pH 1, the maximum hydrolysis of coconut husk extract at 29.21 ± 0.71% was attained after 4 h of incubation. The maximum hydrolysis of CHE at pH 2 and pH 3 is 19.26 ± 0.55% and 18.18 ± 1.45%, respectively, after 5 h of incubation. From the present study, the coconut husk extract was found to be a potential prebiotic candidate as it shows resistant towards the artificial human gastric juice. There are some other potential candidates that having the similar property are reported in the literature, such as, bamboo shoot crude polysaccharides with 0.41 ± 0.05 to 0.66 ± 0.10% hydrolysis for 6 h of incubation [33], kojiooligosaccharides with 0% hydrolysis for 6 h of incubation, which had 100% resistance to artificial gastric acid [34] or gluco-oligosaccharides with 1.6% hydrolysis for 6 h of incubation produced by Gluconobacter oxydans NCIMB 4943 [35].

On the other hand, the inulin hydrolysis of 12.79 ± 1.15%, 1.52 ± 0.03%, 0.91 ± 0.16% and 2.08 ± 0.13% attained at pH of 1, 2, 3 and 4 respectively after two hours of incubation. Although the digestibility of CHE was higher as compared to inulin, there was 76% of the extract was expected to reach the intestine whereas 87.21% of inulin was expected to reach the intestine.
Figure 4. Percentage of hydrolysis (a) coconut husk extract (CHE) and (b) inulin after gastric juice digestion at various pH, Incubated at 37 °C for five hours. Symbols: (■), pH 1; (♦), pH 2; (▲), pH 3; (●) pH 4. Error bars indicate the mean ± standard deviation of three replicates. The error bars were smaller than the size of the symbols as regards to the data points without error bars.

3.5. Fourier Transform Infrared (FTIR) Spectroscopy
Fourier transform infrared (FTIR) spectra of the coconut husk extract (CHE) was performed to identify the main functional groups of extract. Table 2 lists the general assignments of FTIR spectra. The FTIR spectra between 4000 and 650 cm\(^{-1}\) are obtained for the analysis of organic functional group in both coconut husk extract (figure 5(a)) and inulin (figure 5(b)). From the analysis, the FTIR
spectrum of coconut husk extract is similar to the FTIR spectrum of inulin, indicating the similar chemical composition of both compounds.

Table 2. General assignment of FTIR spectra of coconut husk extract (CHE) [36].

| Wavenumber, cm\(^{-1}\) | Experimental FTIR bands, cm\(^{-1}\) | Assignment                      |
|------------------------|-------------------------------------|---------------------------------|
| 3200–3400              | 3278.47                             | vO-H (OH) H bond                |
| 1664–1634              | 1634.24                             | Absorption of water             |
| 1125–1162              | 1160.16                             | vC-Css (C-O-C)                  |
|                        |                                     | glycosidic bonds                |

Figure 5. FTIR spectrum of (a) coconut husk extract (CHE) and (b) inulin.

According to figure 5, there are several dominant bands can be observed, such as ~3200 – 3400, ~2200–2400, ~1550–1650 and ~1000–1200 cm\(^{-1}\). A peak with strong and broad band at the absorption between 3200–3400 cm\(^{-1}\) is corresponded to the hydroxyl (O-H) bond [33]. Meanwhile, there is another peak with absorption around 1630 cm\(^{-1}\) corresponded to the carbonyl group (C=O) bond stretching [37]. This band was assigned with the absorption of water by the substrate [36]. Another
peak at around 2400 cm\(^{-1}\) indicates C-H stretching of CH\(_2\) group. The band at 1160 cm\(^{-1}\) shows the present of C-O-C ring stretching vibration [36].

Overall, the FTIR spectra indicates the coconut husk extract contain hydroxyl group (O-H) group and carbonyl (C=O) group, that are similar to the FTIR spectrum of inulin. The results of present study indicated that both coconut husk extract and inulin contain similar functional groups.

3.6. Nuclear Molecular Resonance (NMR) Spectroscopy

Nuclear molecular resonance (NMR) spectroscopy was conducted to elucidate the structure of coconut husk extract. Figure 6 (a) and (b) show the \(^1\)H-NMR spectra of the coconut husk extract and inulin, respectively.

The \(^1\)H NMR spectrum of the coconut husk extract (CHE) shows the present of signal in the anomeric region at 5.2 ppm. Meanwhile, the broad peak signal at 3.2 to 4.2 ppm is probably due to the presence of other compounds. On the other hand, the \(^1\)H-NMR spectrum of the inulin showed the presence in the anomeric region of two main signals at 5.2 ppm and 5.4 ppm. The signals at 5.2 to 5.4 ppm might due to the presence of vinylic proton [38, 39]. Besides, intense signals were observed at 3.2 to 4.2 ppm. These signals confirmed the identification of the α-Glu unit of free glucose [40].

There are six main signals at 74.04, 94.125, 115.336, 164.299, 165.299 and 189.793 ppm shown in the \(^{13}\)C-NMR spectrum of the CHE (figure 7 (a)). The chemical shift at 74.04 and 94.125 ppm designate the presence of RCH\(_2\)O group. Meanwhile, C=C bond is found to be presence in CHE as there is a signal observed at 115.336 ppm. Furthermore, the signals at 164.299 ppm onwards show the presence of C=O bond in CHE.

On the other hand, the \(^{13}\)C-NMR spectrum of the inulin in this study was compared to the fructooligosaccharides from escarole in the literature [41]. There are two major regions observed for \(^{13}\)C-NMR spectrum of inulin – low frequency region at 60.0 to 78.0 ppm and a region between 80.0 ppm and 105.0 ppm, which were believed to be two carbon signals of anomeric monosaccharides [42].

The characteristic signal at 103.57 ppm corresponds to the carbon involved in β-(2→1)-D-fructose intra-chain binding. The signals at 60.875 ppm and 62.18 ppm were assigned to the methylene group (CH\(_2\)). Another two signals 76.563 ppm and 75.6 ppm might attribute to the signs of the methinic group (CH) [43, 44].
Figure 6. (a) $^1$H-NMR spectrum of coconut husk extract; (b) $^1$H-NMR spectrum of inulin.
4. Conclusion

The coconut husk extract (CHE) was subjected to several prebiotic potency tests in this study. The study's findings suggest that the CHE promotes the growth of probiotic bacteria such as *L. acidophilus*, *L. casei*, and *B. bifidum*. The extract is resistant to *in vitro* enzymatic digestion with a maximum percentage of hydrolysis of 43.70 ± 0.15% at pH 8 after five hours of incubation. The maximum percentage of hydrolysis for *in vitro* gastric juice digestion is 29.21 ± 0.71% at pH 1 after four hours of incubation. These findings suggest that the extract is partially digestible. FTIR spectra indicate that the functional groups, such as the hydroxyl group (O-H) group, carbonyl (C=O) group and C-O-C ring, are available in the CHE. Meanwhile, NMR spectra reveal that CHE has a similar structure to the inulin-type oligosaccharides.

**Figure 7.** (a) $^{13}$C-NMR spectrum of coconut husk extract; (b) $^{13}$C-NMR spectrum of inulin.
In a nutshell, CHE fulfils the key criteria for being considered as a potential prebiotic. It is resistant to gastric juice and enzymatic digestion, and it promotes the growth of the selected probiotic bacteria. CHE also shows a similar structure to the commercially available inulin-type oligosaccharides. Additionally, the results obtained using CHE are comparable to those obtained using commercially available prebiotics inulin. Thus, CHE may be classified as a prebiotic with the potential to be used as a functional food ingredient.

Acknowledgement
The authors would like to acknowledge the University College of Technology Sarawak (UCTS/RESEARCH/1/2019/03; UCTS/RESEARCH/2/2018/10) for research funding and technical support.

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