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Research Article

Characterization of Soluble Polysaccharides from Coconut Residue of Virgin Coconut Oil Production

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ABSTRACT

This study aimed to explore the potential of soluble polysaccharides extracted from defatted coconut residue (DCR) as a prebiotic. Our findings reveal that the defatting process of coconut residue (CR) changes its chemical composition of coconut residue in terms of moisture content (7.04 ± 0.04%), fat (3.99 ± 0.55%), protein (2.93 ± 0.18%), ash (0.76 ± 0.04%), and carbohydrate contents (46.58 ± 1.05%). The percentage of soluble polysaccharide was 2.50 ± 0.40. The extraction of soluble polysaccharides (SP) from DCR resulted in increased total sugar contents and non-reducing sugars. Structural analysis of SP by Fourier transform infrared (FT-IR) analysis showed a predominantly polysaccharide composition. High performance liquid chromatography (HPLC) analysis further identified mannose (27.87%) and glucose (25.00%) as the major monosaccharides present in SP. Remarkably, SP exhibited stronger resistance (92.42% resistance) to acidic condition in the human stomach compared to inulin (25.52% resistance) at pH 1. Furthermore, SP demonstrated prebiotic properties by promoting the growth of *Lactobacillus acidophilus* TISTR 2365. These findings confirmed the promising prebiotic properties of coconut residue after cold-pressed coconut oil processing, which may be used as an ingredient in functional foods and nutraceutical products.

1. Introduction

In the current health food market, particularly in those aimed at boosting the body's immune system against microbial pathogens, there has been a notable increase in the incorporation of prebiotics into these products. Prebiotics are essentially non-digestible food ingredients, mainly dietary fiber, oligosaccharides, and polysaccharides that can substantially affect the consumer health by promoting a healthy balance of beneficial bacteria in the large intestine, thereby enhancing the immune response (Kolida *et al.*, 2002). Prebiotics are not digested in the stomach and small intestine; instead, they enter the large intestine, where they serve as specific food for beneficial bacteria, particularly *Bifidobacteria* and *Lactobacillus*. These bacteria aid in inhibiting harmful bacterial growth (Bamigbade *et al.*, 2022). In 2016, the International Scientific Association for Probiotics and Prebiotics redefined prebiotics as substances that can be selectively used and transformed by the host intestinal flora under the premise that they are beneficial to host health. Therefore, the new definition of prebiotics includes oligosaccharide carbohydrate and non-carbohydrates (Gibson *et al.*, 2017). In the large intestine, prebiotics are fermented to produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. SCFAs influence the structure of the large intestine, provide energy to intestinal cells, reduce pH levels, stimulate water and mineral absorption, and support the influx of beneficial probiotics (Peredo Lovillo *et al.*, 2020). The demand for prebiotics has been on the rise, with the global prebiotics market reaching a value of 7.99 billion US dollars in 2023 (Pulidindi and Ahuja, 2024). Reflecting this trend, the prebiotics markets in Thailand and the Asia Pacific were valued at 73.4 and 2,468 million US dollars in 2023, respectively (Prebiotics market size, 2023). This growth can be attributed to various factors, including increased awareness due to the global

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COVID-19 pandemic situation, which has prompted global populations to pay more attention to health.

The coconut residue (CR) is obtained from the extraction of coconut oil or mechanical squeezing to produce coconut milk. Currently, this remaining coconut residue is primarily used as fertilizer and low-cost animal feed (Sulaiman *et al*., 2013). Typically, coconut residue is a source of carbohydrate, accounting for 43-45% of its composition, particularly in the form of oligosaccharides, such as mannan-oligosaccharides (MOS), along with other sugars like mannose, glucose, galactose, and arabinose (Khuwijitjaru *et al*., 2012). MOS are composed of linear chains of mannose sugars and have attracted considerable interest as prebiotics. MOS extracted from yeast cell wall are effective in enhancing the immune system of animals and are already widely applied in the feed industry (Yamabhai *et al*., 2014). Interestingly, mannan-rich plants including copra meal showed potential as prebiotics and have health-promoting effects including antibacterial properties and the ability to promote the growth of probiotic bacteria in both humans and livestock (Intaratrakul *et al*., 2022; Asbury and Saville, 2025). Nowadays, researchers have recognized the benefits and potential of utilizing discarded coconut residue to produce functional food ingredients rich in dietary fiber. Previous studies have shown that polysaccharide extracts from coconut residue can resist artificial human gastric juices and enhance the growth of *Lactobacillus casei* Shirota and *L. bulgaricus* (Mohd Nor *et al*., 2017).

Therefore, this research focused on utilizing coconut residue, an abundant, fiber-rich by-product derived from the cold-pressed coconut oil extraction process of a community enterprise. The objective is to investigate the prebiotic potential of crude soluble polysaccharides (SP) extracted from defatted coconut residue (DCR), with the aim of advancing its development as a functional food ingredient. The use of coconut residue offers a compelling prospect owning to their cost-effectiveness as a raw material for prebiotic production, thereby enhancing the economic value of coconut residue.

2. Materials and Methods

2.1 Chemical and bacterial strains

Inulin and 3,5-dinitrosalycylic acid (DNS) were obtained from TCI (Tokyo, Japan). Trifluoroacetic acid (TFA) was purchased from Fisher Scientific (PA, USA). α-Amylase from porcine pancreas was obtained from Sigma (MO, USA). De Man, Rogosa, and Sharpe (MRS) broth and agar were obtained from Himedia (Mumbai, India). 98% Sulfuric acid and D-glucose were obtained from Ajax Finechem (Sydney, Australia). All chemicals used were of analytical grade. *Lactobacillus acidophilus* TISTR 2365 was obtained from Thailand Institute of Scientific and Technological Research (TISTR, Thailand).

2.2 Preparation of coconut residue (CR) and defatted coconut residue (DCR)

The coconut residue was obtained from a local community “Takien Tia”, Chonburi, Thailand. The coconut residue was oven-dried at 60 °C for 7 h using a tray dryer (OFM, Thailand). Subsequently, it was ground into powder using an electric grinder (Panasonic, Japan) to obtain coconut residue (CR) powder, which was then passed through a 50-mesh sieve. CR was kept in a sealed plastic bag and stored at room temperature (Thongsook and Chaijamrus, 2018).

To obtain defatted coconut residue (DCR), CR powder was subjected to fat removal by mixing 100 g of dried CR powder with 1 L of hexane for 18 h at room temperature. After hexane removal, the coconut residue was dried in a hot air oven (Memmert, Germany) at 45 °C for 3 h to evaporate any remaining hexane (Raghavendra *et al*., 2004). The DCR was stored in an airtight package for further analysis.

2.3 Proximate analysis of CR and DCR

The coconut residue powder (CR) and defatted coconut residue powder (DCR) samples were subjected to proximate analysis following the AOAC (2002) method. The components analyzed included moisture content, protein, fat, ash, and fiber. Total carbohydrate (% wet basis) was calculated using the following formula:

Total carbohydrate (%) = 100 – [protein content (%) + crude fat (%) + moisture (%) + ash (%) + fiber (%)]

2.4 Extraction of crude soluble polysaccharides using hot water

The extraction process followed the method outlined by Bello *et al*. (2018) with some modifications. Initially, DCR was subjected to extraction using distilled hot water at a ratio of 1:20 w/v at 80 ºC for 2 h. The mixture was subsequently filtered through a cheese cloth. This extraction process was repeated twice to ensure the removal of any residual polysaccharides. The extracts were combined and concentrated using a rotary evaporator to reduce the volume to 1/5 of original. Subsequently, 95% (v/v) ethanol was added four times of the original volume to precipitate the polysaccharides. The mixture was then refrigerated at 4 ºC overnight. Following precipitation, the water-soluble polysaccharide was isolated by centrifugation at 9300 xg for 15 min at 4 ºC. The supernatant was decanted, and the obtained sample was subjected to freeze-drying to obtain a dry sample. Finally, the dried sample was stored at room temperature for further analysis.

2.5 Determination of total sugar content, reducing sugar, and non-reducing sugar

2.5.1 Determination of total sugar content

Total sugar content was assessed using the phenol-sulphuric method as described by Dubois *et al*. (1956). Initially, 2 mL of the sample solution was combined with 5% (v/v) phenol and 5 mL of concentrated 98% sulfuric acid (H_2SO_4). The mixture was vortexed and incubated at room temperature for 30 min. Subsequently, the absorbance of solution was measured at 490 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Total sugar content was determined based on a standard curve generated using D-glucose and expressed as mg/g extract.

2.5.2 Determination of reducing sugar and non-reducing sugar

The reducing sugar assay was performed following the method described by Robertson *et al*. (2001). One milliliter of the sample was mixed with 3,5-dinitrosalicylic acid (DNS) reagent and boiled for 5 min. Subsequently, the sample was allowed to cool to room temperature for 5 minutes before adding 7 mL of distilled water. The absorbance was then measured at 540 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Total reducing sugar was calculated with D-glucose as a standard and expressed as mg/g extract. Non-reducing was calculated using the following formula:

Non-reducing sugar (mg/g) = Total sugar content (mg/g) – Reducing sugar content (mg/g)

2.6 Analysis of structural properties of soluble polysaccharides

The structure of soluble polysaccharides (SP) was determined using the Fourier transform infrared (FT-IR) spectroscopy. Infrared spectra were acquired using a Nicolet 6700 FT-IR spectrum analyzer (Thermo Scientific, USA) over the range of 4000-400 cm^{-1}. Samples were prepared by grinding 1 mg of SP with potassium bromide (KBr) and pressing the mixture into a pellet for FT-IR analysis (Mohd Nor *et al*., 2017).

2.7 Analysis of monosaccharide composition of soluble polysaccharides

Soluble polysaccharides (SP) were subjected to hydrolysis prior to monosaccharide determination. In brief, 10 mg of SP was dissolved in 10 mL of 2M trifluoroacetic acid (TFA) and incubated at 95 ºC for 6 h. The resulting mixture was then centrifuged at 9300 xg for 5 min. The supernatant was collected and evaporated using a rotary evaporator at 60 ºC to remove TFA. The hydrolysate was dissolved in 1 mL of distilled water. High-performance liquid chromatography (HPLC) equipped with a refractive index detector (Hitachi, Japan) was used to analyze the monosaccharide composition. The HPLC system employed with a BP-800 Ca column, with HPLC-grade water serving as the mobile phase, pumped at a flow rate of 0.6 mL/min at 85 ºC for 20 min. A 10 µL injection volume was used for each sample. The concentration of each sugar was determined by comparing peak areas with those of standard sugars, including glucose, fructose, rhamnose, mannose, and xylose.

2.8 Determination of prebiotic activity of soluble polysaccharides on Lactobacillus acidophilus TISTR 2365

The prebiotic effect of SP was evaluated through the *L. acidophilus* TISTR 2365 growth. MRS medium was used as a basal growth medium. *L. acidophilus* TISTR 2365 was cultured at 35°C with samples of SP at concentrations of 0.1 %. Following incubation for 0, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h at 35 ºC, sampling was collected. The total plate count was determined using MRS agar for enumeration. Plates were incubated at 35 °C for 48 h. The number of bacteria were counted, calculated, and expressed as log CFU/mL (Torshabi *et al*., 2023).

2.9 Determination the effect of artificial human gastric juice on hydrolysis of soluble polysaccharides

The effect of artificial human gastric juice on hydrolysis of SP was determined as described by Korakli *et al*. (2002). Initially, SP was dissolved in distilled water to make a concentration of 1 % (w/v). Artificial human gastric juice was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 8.25 g of $Na_2HPO_4.2H_2O$, 14.35 g of $NaHPO_4$, 0.1 g of $CaCl_2.2H_2O$, and 0.18 g of $MgCl_2.6H_2O$ in distilled water to make a 1 L solution. The pH of the solution was adjusted to 1, 2, 3, 4, and 5 using 6 M HCl, respectively. For the hydrolysis assay, 5 mL of the solution at each pH level was mixed with 5 mL of SP sample. The mixture was then incubated in a water bath at 37 °C for 6 h. Samples were collected at 0, 30 min, 1, 2, 3,4, 5, and 6 h to determine the total and reducing sugar contents. Inulin was used as a positive control for comparison. The percentage of hydrolysis degree was calculated using the following formula:

$$\text{Hydrolysis degree (\%)} = \frac{\text{Reducing sugar x 100}}{\text{Total sugar content-initial reducing sugar content}}$$

2.10 Statistical analysis

Each experiment was performed three times and the results were reported as ± standard deviation (SD). Data were analyzed by one-way ANOVA using SPSS statistics 17.0 (SPSS Inc., Chicago, IL, USA). All statistics were based on a confidence level of 95 %, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Chemical composition of coconut residue (CR) and defatted coconut residue (DCR)

The chemical composition of CR and DCR is presented in Table 1. Carbohydrate and fiber were identified as the major components in both CR and DCR. The defatting process resulted in a significant reduction in fat content in coconut residue ($p < 0.05$), decreasing from 16.35 ± 0.36 % in CR to 3.99 ± 0.55 % in DCR. Similarly, there was a notable decrease in protein content from 4.56 ± 0.32 % CR to 2.93 ± 0.18 % in DCR. Additionally, the ash content in DCR (0.76 ± 0.04 %) was found to be lower than that in CR (1.24 ± 0.01 *%).* The extraction of fat from coconut residue resulted in changes in its chemical composition, including lower fat, protein, and ash contents, while the carbohydrate content was higher compared to CR. Specifically, the fat content of DCR in this study was significantly lower than the findings reported by Mohd Nor *et al*. (2017), who observed a fat content of 17.26 % in defatted coconut residue. The difference in lipid contents might be due to the variety of coconut, growing conditions, and processing methods.

Hot water extraction followed by precipitation with alcohol is the most widely used method for extracting polysaccharides (Sritrakul and Keawsompong, 2021; Gunarathne *et al*., 2024). Soluble polysaccharides (SP) were obtained from DCR as a water-soluble white water-soluble as shown in Figure 1. In this study, the percentage yield of SP was 2.50 ± 0.40 which exceeded that reported by Abbasiliasi *et al*. (2019) (% yield: 1.33 ± 0.30) and Mohd Nor *et al*. (2017) (% yield: 0.73 ± 0.04). The variation of extraction yield may be attributed to several factors, including the extraction method employed, the ratio of water to raw material, extraction duration, and extraction temperature (Cai *et al*., 2008).

A B C

Figure 1 A) Coconut residue (CR) B) Defatted coconut residue (DCR) and C) Soluble polysaccharides (SP)

3.2 Total sugar content, reducing sugar, and non-reducing sugar

As shown in Table 2, the removal of fat from CR resulted in a remarkable increase in the total sugar content of DCR, rising from 63.31 ± 7.39 mg/g to 85.77 ± 5.71 mg/g. Furthermore, the percentage yield of soluble polysaccharides (SP) obtained in this study was 4.40 ± 0.11 %. Subsequently analysis revealed that the total sugar content of SP was 397.29 ± 66.24 mg/g, with a reducing sugar content of 27.47 ± 0.67 mg/g. Accordingly, the non-reducing sugar content of SP was calculated to be 369.82 ± 66.42 mg/g. Remarkably, SP exhibited the highest content of both total sugar and non-reducing sugar ($p < 0.05$) compared to other samples. The extraction of soluble polysaccharides from DCR had the highest proportion of non-reducing sugars. This observation suggests that the SP may contain a higher proportion of oligosaccharides or polysaccharides compared to monosaccharides. The determination of non-reducing sugar is indicative of the presence of oligosaccharides (Thungchoho, 2012).

3.3 Monosaccharides content of soluble polysaccharides

The monosaccharide composition of soluble polysaccharides (SP) comprises two major sugars: mannose, accounting for 27.87 % and glucose, comprising 25.00 %. Fructose also presented a smaller proportion of 7.10 %. This result agrees with the study by Thongsook and Chaijamrus (2018), who found that glucose and mannose were the major sugars detected in the copra meal hydrolysis. The study on extracted polysaccharides by Sritrakul and Keawsompong (2021) reported that mannose was the main sugar accounting for 99 % of total monosaccharides. A study reported that copra meal is a main source of galactomannan (Rungruangsaphakun and Keawsompong, 2018). Other previous studies showed that the composition of polysaccharides in coconut residue was glucose (1.66 %) and fructose (19.16 %) (Mohd Nor *et al*., 2017). It was revealed that geographical area and seasonal harvest of the plant sources could contribute to the variation in the monosaccharide composition (Ng *et al*., 2010).

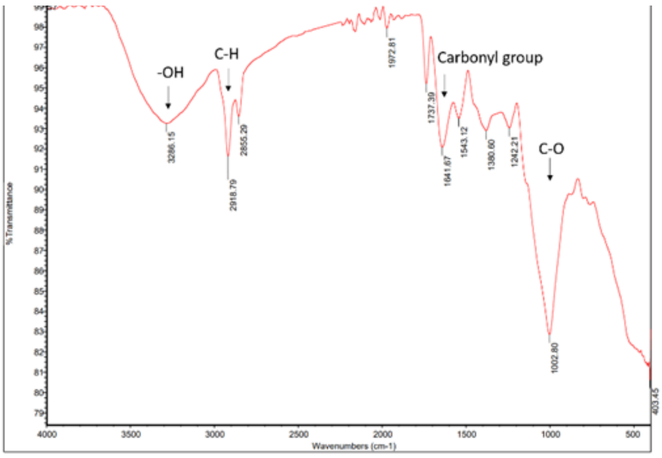

Figure 2 FTIR spectrum of soluble polysaccharides

3.4 FT-IR Analysis of SP

Combining data from chemical analysis with FT-IR spectroscopy allows for the approximate identification of compounds as shown in Figure 2. In this study, a broad band centered at 3286.15 cm-1 was attributed to hydroxyl (OH) group, while the band observed at 2918.79 cm^{-1} indicated C-H stretching, suggesting the presence of polysaccharides. Additionally, a band in the region of 1641.67 cm^{-1} represented the stretching vibration of the carbonyl group. Adsorption at 1002.80 cm^{-1} indicated CO stretching vibration. Taken together, these findings indicate that SP is mainly composed of polysaccharides.

3.5 Effect of artificial human gastric juice on hydrolysis of soluble polysaccharides

According to the requirement for prebiotic products, it is essential that they are not hydrolysed under acidic conditions to ensure their passage to the intestine where they can facilitate the proliferation of beneficial gut bacteria (probiotics). SP containing non-reducing sugar exhibits characteristics indicative of an oligosaccharide with potential prebiotic characteristics. To assess its resistance to hydrolysis, SP and inulin (used as a positive control) were subjected to incubation with artificial human gastric juice at various pH levels (Figure 3). For inulin, the degree of hydrolysis after 5 h of incubation at pH 1, 2, 3, 4, and 5 were 74.48, 10.65, 0.45, 0.42, and 0.16 %, respectively (Figure 3A). For SP, the degree of hydrolysis at pH 1, 2, 3, 4, and 5 were 7.58, 9.58, 6.86, 6.81, and 0.60 %, respectively (Figure 3B). Notably, at pH 1 (lowest pH), the degree of hydrolysis of SP was lower compared to inulin. The hydrolysis of polysaccharides with human gastric juice was influenced by their monosaccharide compositions, ring configurations, and types of glycosidic linkages (Wang *et al*., 2015). In this study, the main composition of SP was mannose. A previous study has reported that a high mannose content could resist digestive enzymes (Asano *et al*., 2003). In addition, a pH level below 2 has been reported to degrade inulin and lead to an increase in reducing sugar due to the β-(2-1) glycosidic bonds in inulin being susceptible to acid hydrolysis (Lin *et al*., 2024). In the present study, at pH 2, no significant differences of acid stability were observed between SP (~90% resistance) and inulin (~89% resistance). Therefore, at pH 2, both SP and inulin were hydrolyzed slowly, and their structures remained relatively intact. Furthermore, the degree of hydrolysis of SP decreased with increasing pH from 1 to 5. It was found that soluble polysaccharides can resist simulated human gastric juice.

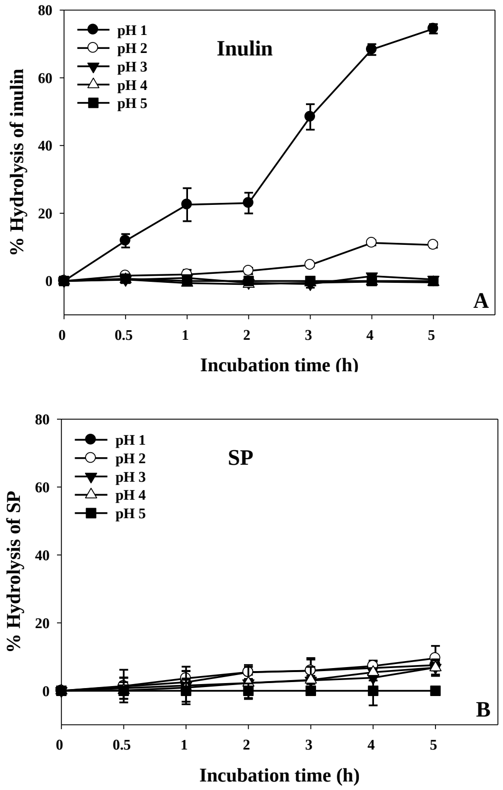

Figure 3 Hydrolysis of inulin (A) and SP (B) after treatment with artificial human gastric juice

In this study revealed that both soluble polysaccharides (SP) and inulin exhibited a higher degree of hydrolysis at low pH compared to high pH. The increased hydrolysis observed at low pH can be attributed to the stomach. Under these acidic conditions, the glycosidic linkages in the polysaccharides are more susceptible to rupture, leading to partial hydrolysis. Consequently, the polysaccharides may degrade into smaller molecules. Prolonged incubation times in acidic conditions further caused the hydrolysis of polysaccharides to monosaccharides or disaccharides (Mohd Nor *et al*., 2017; Wang *et al*., 2015). The digestibility of polysaccharides during the initial two-hour period was a high degree of hydrolysis at pH 1 and 2. The percentage of SP that resisted human gastric digestion at pH 1 was approximately 92 %, which markedly exceeded that of inulin (25 %). It might be due to the differences in their monosaccharide compositions. SP predominantly consists of mannose, a monosaccharide that has been reported to exhibit resistance to digestive enzymes (Asano *et al*., 2003). Conversely, inulin is primarily composed of fructose molecules (Prosky, 1999). Consequently, there is a possibility that most of the SP is not digested, enabling it to transit to the large intestine where it can serve as a substrate for native probiotics.

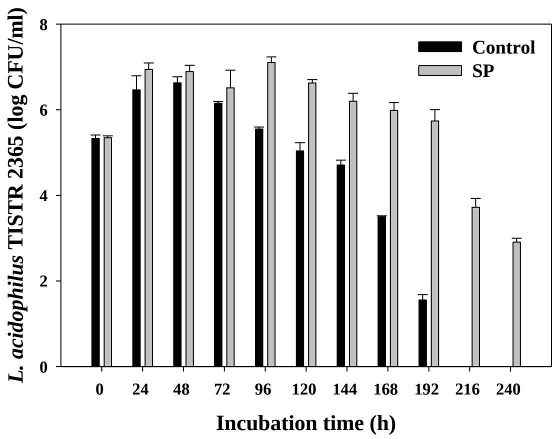

Figure 4 Proliferation of *L. acidophilus* TISTR 2365 on SP

3.6 Prebiotic activity of SP on Lactobacillus acidophilus TISTR 2365

Figure 4 depicts the ability of 0.1 % SP to promote the growth of probiotic bacteria, *Lactobacillus acidophilus* TISTR 2365, following incubation periods ranging from 0 to 240 h. The concentration of prebiotics is also an important factor affecting the production of short-chain fatty acids (SCFAs) by probiotics. The increase of SCFAs leads to a decrease in the pH of the intestine and is beneficial to human health (You *et al*., 2022). In this study, using 0.1 % SP powder is an optimal concentration to lower intestinal pH and promote the growth of *Lactobacillus* acidophilus TISTR 2365.

A significance difference ($p < 0.05$) was observed between the control group (MRS only) and MRS supplemented with SP. Specifically, the addition of SP was found to enhance the growth of *L. acidophilus* TISTR 2365 compared to the control group.

L. acidophilus was able to utilize various oligosaccharides and polysaccharides (Dong *et al*., 2024). In the control group, the cell count decreased after 96 h, whereas in the SP treatment, the population of *L. acidophilus* TISTR 2365 remained higher. Following nutrient depletion in the MRS medium, SP served as an additional carbon source. As a result, the decline in cell counts was delayed until 240 h when compared with the control. The enhanced proliferation of probiotics in SP-supplemented media may be attributed to the sugar content present in polysaccharides, including mannose, glucose, fructose, which serve as substrates for bacterial growth. Furthermore, soluble polysaccharides are more effective for bacterial utilization. These polysaccharides can be readily hydrolyzed by bacterial enzymes, releasing sugars that can be metabolized by probiotics and become more beneficial for gut health (Mohd Nor *et al*., 2017).

Table 1 Chemical composition of coconut residue (CR) and defatted coconut residue (DCR)

Parameters	Composition (%)	
	Coconut residue (CR)	**Defatted coconut residue (DCR)**
Moisture content	4.51 ± 0.21[a]	7.04 ± 0.04[b]
Crude protein	4.56 ± 0.32[b]	2.93 ± 0.18[a]
Crude fat	16.35 ± 0.36[b]	3.99 ± 0.55[a]
Ash content	1.24 ± 0.01[b]	0.76 ± 0.04[a]
Crude fiber	39.31 ± 0.96a	38.70 ± 1.48[a]
Carbohydrate	34.03 ± 1.51a	46.58 ± 1.05[b]

Table 2 Total sugar, reducing sugar, and non-reducing sugar of CR, DCR, and SP

Samples	**Total sugar (mg/g)**	**Reducing sugar (mg/g)**	**Non-reducing sugar (mg/g)**
CR	63.31 ± 7.39[a]	48.50 ± 4.23[c]	14.81 ± 5.08[a]
DCR	85.77 ± 5.71[b]	19.69 ± 2.77[a]	66.08 ± 2.98[b]
SP	397.29 ± 66.24[c]	27.47 ± 0.67[b]	369.82 ± 66.42[c]

4. Conclusion

The soluble polysaccharides (SP) from defatted coconut residue were obtained. These findings confirm the potential of SP as a prebiotic ingredient due to its resistance to gastric digestion and its subsequent availability for fermentation by gut microbiota in the colon. Therefore, this study highlights the sustainability aspect of utilizing coconut residue, which encourages the efficient use of agricultural waste and enhances economic feasibility. This contributes to its significant potential for large-scale commercial applications. Further study to confirm the characteristics of MOS could be performed by molecular weight distribution analysis using HPAEC-PAD, linkage type analysis by methylation followed by GC-MS and NMR, and evaluation of prebiotic activity through human fecal fermentation.

5. Acknowledgment

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