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RESEARCH

Subcritical Fluid Process for Producing Mannooligosaccharide-Rich Carbohydrates from Coconut Meal and Their In Vitro Fermentation

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Abstract

This work aimed to produce mannooligosaccharide-rich carbohydrates from coconut meals for food application by using environmentally friendly processes. Coconut meal from coconut milk manufacturing was defatted by subcritical ethanol extraction at 100 °C before hydrolysis in subcritical water at different temperatures (120−230 °C), followed by ethanol precipitation. The obtained ethanol-insoluble precipitates contained mainly carbohydrates (89.09–98.88%) with small amounts of protein (0–5.33%) and 5-hydroxymethyl-2-furaldehyde (0–13.16 mg/100 g). The molecular weights of the final product were in the range of 0.83–34.70 kDa. The highest yield of the final product was from the treatment at 220 °C (10.76 g/100 g defatted coconut meal), while the treatment at 230 °C possessed the highest content of mannooligosaccharides with a degree of polymerization of 2–6 (46.12%). The obtained carbohydrates showed antioxidant capacities and enhanced in vitro growth of lactobacilli and bifidobacteria. Acetic and propionic acids were the major short-chain fatty acids formed by fermentation.

Keywords Coconut meal · Subcritical water treatment · Mannooligosaccharides · Prebiotics · Functional food ingredient

Introduction

Prebiotics are defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). The market for prebiotics has been continually growing, and prebiotics have been increasingly used by the food industry as functional ingredients due to their health promotional effects (Farias et al., 2019). Although there are many types of prebiotics, most of them are a subgroup of carbohydrates, especially oligosaccharides (Davani-Davari et al., 2019). Fructans (fructooligosaccharides) and galactans (galactooligosaccharides) are widely accepted as prebiotics for human consumption (Gibson et al., 2017).

Mannooligosaccharides can be produced from several mannans containing plant or agricultural waste, such as palm kernel meal, spent coffee ground, and coconut meal. Many studies have confirmed that mannooligosaccharides could be potentially utilized as prebiotics because they are non-digestible and can be fermented by human intestinal beneficial microflora, especially bifidobacteria and lactobacilli (Singh et al., 2018). Moreover, mannooligosaccharides showed a potential use as a functional food additive for specific health effects such as anti-allergic agents (Ozaki et al., 2007), anti-cancer, anti-inflammatory, reduction of serum triacylglycerols, and immuno-modulating properties (Jana et al., 2021).

One of the potential raw materials for mannooligosaccharides is coconut kernel. The kernel is used for producing coconut milk and oil. The coconut meal, which is obtained from coconut milk production as a by-product, contains about 45−70% of carbohydrate components, 20% crude fat, and a small amount of protein (Khuwijitjaru et al., 2012). The carbohydrate in coconut meal is mainly composed of mannan polymers (61%), while other polysaccharides are cellulose, arabinoxylogalactan, galactomannan, arabinomannogalactan, and galactoglucomannan (Saittagaroon et al., 1983). However, the meal has been mainly used as feed, which is relatively low value, and therefore utilizing this by-product as a functional ingredient for human consumption should be studied.

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Subcritical water treatment is an environmentally friendly technique with a wide range of applications. Subcritical water treatment has been majorly studied as a promising method for extracting phenolic and antioxidant compounds from various raw materials (Jo et al., 2013; Karacabey et al., 2012; Lin et al., 2022; Pavlić et al., 2016; Song et al., 2018; Zheng et al., 2019), while depolymerization of polysaccharides in subcritical water to produce oligosaccharides has been also reported (Gomes-Dias et al., 2022; Ruthes et al., 2017). However, due to the different microstructures of the raw materials and chemical structures of polysaccharides, the hydrolysis performance by subcritical water may vary. Khuwijitjaru et al. (2014) showed that subcritical water treatment could hydrolyze the polysaccharides in coconut meal into mannose and oligosaccharides. Although several studies showed the prebiotic properties of mannooligosaccharides obtained from coconut meals, most of them focused on enzymatic hydrolysis (Ghosh et al., 2015; Intaratrakul et al., 2022; Jana & Kango, 2020; Pangsri et al., 2015; Rungruangsaphakun & Keawsompong, 2018; Suryawanshi & Kango, 2021; Thongsook & Chaijamrus, 2018; Titapoka et al., 2008).

Therefore, the objectives of this study were to improve the production of mannooligosaccharides from coconut meals using a novel combination of environmentally friendly methods. In this study, subcritical ethanol extraction was employed to remove fat from the coconut meal. Then, the mannooligosaccharides were obtained from the defatted coconut meal using subcritical water treatment at various temperatures. In addition, following ethanol precipitation to obtain mannooligosaccharide-rich carbohydrates, their physicochemical properties, antioxidant properties, and in vitro fermentation were investigated to ascertain the usefulness of the obtained mannooligosaccharides as a functional food ingredient.

Materials and Methods

Defatted Coconut Meal

Freshly produced coconut meal (Vara Food and Drink Co. Ltd., Nakhon Pathom, Thailand) was obtained on the day of production. It was dried in a hot-air oven and sieved to a particle size of 0.3–2.0 mm. Defatting was performed using subcritical ethanol extraction as described by Plangklang et al. (2021). Briefly, the dried coconut meal (10 g) was mixed with 80 mL of absolute ethanol (RCI Labscan, Bangkok, Thailand) in a batch-type reactor (120 mL net volume, Taiatsu Techno Corporation, Osaka, Japan) and extracted at 100 °C for 45 min. The vessel was cooled to prevent an undesirable reaction in an ice-water bath. The treated mixture was filtered through a Whatman No. 1 paper. The defatted coconut meal was dried in a hot-air oven at 60 °C for 6 h and kept at −18 °C. A total of 150 batches of the defatted meal were prepared and pooled together. Its moisture content, crude protein, crude fat, and ash were determined using AOAC (2000) methods and their values were 5.47, 3.10, 4.02, and 1.12% (wet basis, w.b.), respectively. Carbohydrate content was determined from the difference to be 86.29% (w.b.).

Chemicals

Mannose, glucose, 5-hydroxymethyl-2-furaldehyde (5-HMF), dextran, 2,2-diphenyl-1-picrylhydrazyl (DPPH), albumin from bovine serum (BSA), 2,4,6-tripyridyl-s-triazine (TPTZ), and Trolox were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standard 1,4-β-D-mannooligosaccharides (mannobiose, mannotriose, mannotetratose, mannopentaose, and mannohexaose) were purchased from Megazyme (Wicklow, Ireland). Folin-Ciocalteu reagent, sulfuric acid, and phenol were purchased from Merck (Darmstadt, Germany). Absolute ethanol (99.5%) was purchased from RCI Labscan (Bangkok, Thailand). Food grade inulin from chicory root (FIBRULINE, 91.7% inulin) was obtained from Cosucra (Warcoing, Belgium). Other reagents and solvents were of analytical grade.

Bacterial Strains and Media

Lactobacillus plantarum (TISTR 1265), *Lactobacillus rhamnosus* (TISTR 2443), *Bifidobacterium bifidum* (TISTR 2129), and *Bifidobacterium breve* (TISTR 2130) were from Thailand Institute of Scientific and Technological Research (Bangkok, Thailand) and MRS media was purchased from Himedia (Nashik, India).

Subcritical Water Treatment

The defatted coconut meal was treated with subcritical water in a batch-type reactor (same as described above) using the 5 g defatted coconut meal and 80 g distilled water. The mixture was heated to different temperatures in the range of 120−230 °C (Supplementary Fig. S1) using a pipe heating blanket (50 × 200 mm, 200 W, Heater Engineer, Tokyo, Japan) with a temperature controller (TXN 700B, As One, Osaka, Japan) connected with a type-K thermocouple to measure the temperature inside the vessel. After heating, the vessel was immediately cooled to stop the reaction using an ice-water bath. The treated mixture was filtered through a Whatman No. 5 paper. The hydrolysate was freeze-dried (LyoQuest-85, Telstar, Terrassa, Spain) and stored at −18 °C until use. All subcritical water treatments were performed in triplicate.

The yields of the hydrolysate and residue were calculated by the ratio of the dry weight of hydrolysate powder (W_H) or residue (W_R) to the dry weight of the defatted coconut meal (W_{DM}) according to Eqs. (1) and (2), respectively.

$$Hydrolysate\ yield\ (\%) = \frac{W_H}{W_{DM}} \times 100 \quad (1)$$

$$Residue\ yield\ (\%) = \frac{W_R}{W_{DM}} \times 100 \quad (2)$$

Ethanol Precipitation

Because only a small amount of the dry hydrolysate was obtained from a single batch, depending on the amount of obtained sample, 5−15 batches of the hydrolysate samples were prepared and pooled as one replication. Triplicated treatments were conducted. The dried hydrolysate was re-dissolved in distilled water to prepare a 10% w/v solution, then left at room temperature overnight and centrifuged at 10,000 × *g*, 4 °C, for 20 min to discard insoluble matter. The supernatant (10 mL) was mixed with absolute ethanol (90 mL) to achieve the final ethanol concentration of 90% v/v (Klinchongkon et al., 2019). The mixture was kept in a refrigerator (4 ± 1 °C) for 24 h, then centrifuged at 10,000 × *g*, 4 °C, for 20 min. The precipitated carbohydrate was collected, freeze-dried, and stored at −18 °C for further analyses.

Color

The color of the lyophilized hydrolysate and precipitate was measured using a colorimeter (NR-12, Nippon Denshoku, Tokyo, Japan) and expressed as lightness (L^*) and hue angle ($h°$).

Total Carbohydrate and Reducing Sugar Contents

The total carbohydrate content was determined using the phenol–sulfuric method (Chaplin & Kennedy, 1986). Two hundred microliters of the sample solution was mixed with 600 µL of 5% w/v phenol solution and 3 mL of sulfuric acid. The mixture was thoroughly mixed by a vortex mixer and allowed to stand for 1 h at room temperature. The absorbance of the mixture was determined at 495 nm using a UV–vis spectrophotometer (Genesys 10 s, Thermo Scientific, Waltham, MA, USA).

The reducing sugar content was determined using the dinitrosalicylic acid method (Chaplin, 1986). Dinitrosalicylic acid solution was prepared by mixing 0.25 g dinitrosalicylic acid and potassium sodium tartrate (75 g in 50 mL of 2 M NaOH), after which the solution volume was adjusted to 250 mL using distilled water. The sample solution (0.2 mL) was mixed with dinitrosalicylic acid solution (2 mL) in a test tube with a screw cap and placed in boiling water for 10 min then cooled in ice water. The absorbance at 540 nm was recorded using the UV–vis spectrophotometer. Mannose was used for preparing standard curves for both analyses.

Protein Content

Protein concentration was determined using the Bradford assay, modified by Ching et al. (2015). One milliliter of Bradford reagent was mixed with 1 mL of sample solution and left at room temperature for 5 min. The absorbance at 595 nm was measured with the UV–vis spectrophotometer. A protein standard curve was constructed using 2.0–10.0 µg/mL of BSA.

5-Hydroxymethyl-2-furaldehyde Content

The 5-HMF content was determined using a high-performance liquid chromatograph (HPLC) (Shimadzu, Kyoto, Japan) (Khuwijitjaru et al., 2014) which comprised an LC-20A pump and a photodiode array detector (SPD-M20A) and equipped with an Inertsil ODS-3 column (4.6 mm I.D. × 150 mm, GL Sciences, Tokyo, Japan). The mobile phase was a mixture of water and methanol in a ratio of 90:10 v/v at a flow rate of 1.0 mL/min. The sample solution was filtered through a 0.45 µm syringe filter, and the injection volume was 20 µL. 5-HMF was monitored at 285 nm. A calibration curve of the 5-HMF standard was prepared for quantification.

Mono- and Oligosaccharide Determination

Mono- and oligosaccharides in the obtained hydrolysate and precipitate were determined by HPLC (Klinchongkon et al., 2019). The sample was re-dissolved with distilled water, after which the solution was mixed with acetonitrile in a ratio of 35:65 v/v and filtered through a 0.45-µm syringe filter. The filtered sample (20 µL) was injected into the HPLC equipped with a refractive index detector (RID-20A, Shimadzu). The separation of oligosaccharides was achieved on a COSMOSIL Sugar-D column (4.6 mm I.D. × 250 mm, Nacalai Tesque, Kyoto, Japan) which was maintained at 35 °C in a column heater (Fortune Scientific, Bangkok, Thailand). A mixture of distilled water and acetonitrile in a ratio of 35:65 v/v was used as the mobile phase at a flow rate of 1.0 mL/min. Mannose, mannobiose, mannotriose, mannotetratose, mannopentaose, and mannohexaose were used for quantification of the mono- and oligosaccharides with a degree of polymerization (DP) range of 2−6.

Molecular Weight Analysis

The molecular weight of carbohydrate in the precipitate and the commercial inulin was determined using size-exclusion chromatography as described by Klinchongkon et al. (2017) with some modifications. The precipitates were re-dissolved in 50 mM of sodium phosphate buffer containing 0.4 M NaCl and 0.02% w/v NaN_3 at pH 7 overnight at ambient temperature and then filtered through a 0.22-µm nylon syringe filter. The sample solution was analyzed using an HPLC equipped with a RID-20A refractive index detector and size-exclusion chromatography column (TSKgel SuperAWM-H, 6.0 mm I.D.×150 mm; Tosoh Corporation, Tokyo, Japan). The buffer solution was also used as a mobile phase at a flow rate of 0.6 mL/min at 35 °C. Glucose (180 Da) and dextran standards (5, 25, 150, 410, and 670 kDa) were used to prepare a calibration curve. The weight-average molecular weight (M_w) was determined using Eq. (3)

$$M_w = \frac{\sum(H_i \times W_i)}{\sum H_i} \tag{3}$$

where H_i is the signal height of each point on the chromatogram measured from the baseline and W_i is the molecular weight of each point on the chromatogram according to the prepared calibration curve.

Antioxidant Activity

Antioxidant activity of the precipitate was measured based on DPPH radical scavenging activity and ferric reducing power (FRAP). DPPH free radical scavenging assay was measured by the procedure described by Molyneux (2004) with some modifications. Briefly, 100 µL of the sample solution was mixed with 3.9 mL of 0.08 mM solution of DPPH in methanol and allowed to stand for 30 min in the dark. The absorbance of mixtures was measured at 517 nm using the UV–vis spectrophotometer.

FRAP assay was performed according to the method modified by Kim and Lee (2009). Briefly, 100 µL of the sample solution was mixed with 2 mL of FRAP reagent, which contained 10 mL of a 10 mM TPTZ solution in 40 mM HCl, 10 mL of 20 mM $FeCl_3.6H_2O$, and 100 mL 0.3 mM acetate buffer (pH 3.6). The mixture was allowed to react in the dark for 30 min, and the absorbance was measured at 593 nm using the UV–vis spectrophotometer.

Trolox solutions were used to prepare the calibration curves for both analyses, and the results were expressed as mmol Trolox equivalent (TE)/g precipitate, d.b.

In Vitro Fermentation

The lyophilized cultures were activated in an MRS medium (pH 6.8) at 37 °C for 12 h. In vitro fermentation of the precipitate (oligosaccharide-rich carbohydrate) obtained from defatted coconut meal was performed using batch fermentation with individual culture. The obtained precipitates and commercial inulin were dissolved in distilled water and passed through a 0.22 µm sterile syringe filter and then added to sterile MRS broth for use as a carbon source at a final concentration of 0.2% w/v. Thereafter, the activated culture (approximately 9 log CFU/mL) was inoculated into the medium at 1% v/v and incubated at 37 °C for 48 h. For *B. bifidum* (TISTR 2129) and *B. breve* (TISTR 2130), the MRS medium was supplemented with 0.05% w/v cysteine, and incubation was conducted in an anaerobic jar. MRS medium without sugar was used as a control. The microbial growth on different carbohydrates was determined by measuring the absorbance at 600 nm (ΔA_{600}) (Jana & Kango, 2020) using the UV–vis spectrophotometer as shown in Eq. (4).

$$\Delta A_{600} = A_{48} - A_0 \tag{4}$$

where A_{48} and A_0 are absorbances of fermented broth at 600 nm after incubation for 48 and 0 h, respectively.

Quantification of Short-Chain Fatty Acids and Lactic Acid

Short-chain fatty acids and lactic acid of the fermentation broth after centrifugation at 10,000×g, 4 °C for 5 min and filtering with a 0.22 µm syringe filter (Andersson & Hedlund, 1983) were analyzed by HPLC using ReproGel H column (8.0 mm I.D.×300 mm, 9 µm, Dr. Maisch, Ammerbuch, Germany) with a PDA detector. The mobile phase was 0.9 mM H_2SO_4 used at a flow rate of 1 mL/min. The column was temperated at 40 °C. The injection volume was 20 µL. Short-chain fatty acids and lactic acid were monitored at 210 nm, and standard curves of formic, acetic, propionic, butyric, and lactic acids were prepared for quantification.

Statistical Analysis

The experiments were conducted in triplicate. The data were analyzed by analysis of variance (ANOVA) using PASW Statistics for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to determine significant differences between means at a significance level of 0.05. Dunnett's test was also used to determine significant differences between the control and other treatments at a significance level of 0.05.

Results and Discussion

Subcritical Water Treatment of Defatted Coconut Meal

Figure 1A and B show the yields of the residue and hydrolysate and the pH of the hydrolysate obtained at different treatment temperatures, respectively. At low treatment temperatures (120 and 140 °C), the yields of the hydrolysate were approximately 4%, while the residue yields were approximately 95%. The yields of hydrolysate gradually increased from 7.36 to 28.77% at temperatures from 160 to 220 °C and then increased rapidly to 60.19% when the temperature was increased to 230 °C. On the other hand, the yields of the residue decreased with the temperature. This suggested that the solid structure of the coconut kernel was hydrolyzed into smaller molecules and dissolved in water throughout the treatment. The initial pH of the mixture was approximately 5.72. With the rising reaction temperature, the pH gradually decreased from 5.57 at 120 °C to 3.42 at 230 °C, indicating the decomposition of components into acidic products such as levulinic and formic acids through the dehydration reaction of monosaccharides (Rasmussen et al., 2014) and also acetic and glycolic acids from the Maillard reaction between reducing sugars and amino acids available in the sample (Hemmler et al., 2018).

It shows in Fig. 1C that lightness (L^*) and hue angle ($h°$) of the hydrolysate powders decreased with treatment temperature from 87 to 16 and 84 to 39, respectively, which corresponded to the color of the hydrolysates which changed from light yellow to dark brown. Kodama et al. (2015) also reported that the color of barley grain extracts obtained by subcritical water treatment changed with the temperature from slightly yellow to dark yellow. These changes in the color of the hydrolysate were attributed to the formation of the products from browning reactions such as the Maillard reaction or the dehydration reaction of sugars.

Figure 1D represents the chemical properties of the hydrolysate obtained from different treatment temperatures, including total carbohydrate, protein, reducing sugar, and 5-HMF contents. At 120–160 °C, increasing temperatures led to a slight increase in the total carbohydrate and reducing sugar contents. However, an increase in temperature to 190 °C and above significantly increased the total carbohydrate and reducing sugar contents, and both components attained the highest contents at 230 °C (91.57 and 81.18 g/100 g hydrolysate, respectively) ($p \leq 0.05$), indicating the extensive hydrolysis of polysaccharides into soluble

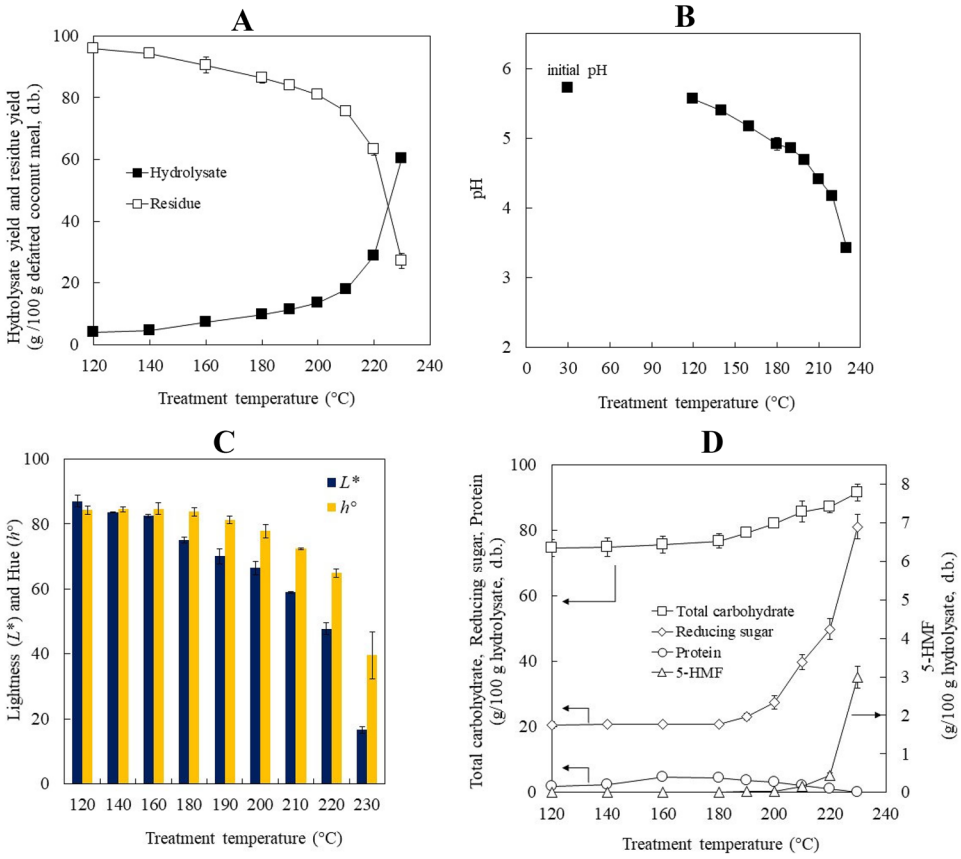

Fig. 1 Effect of subcritical water treatment temperature on yields of hydrolysate and residue (**A**), pH of liquid hydrolysates (**B**), color of hydrolysate powders (**C**), and chemical properties of hydrolysate powders (**D**)

monosaccharides, oligosaccharides, and smaller polysaccharides, at high treatment temperatures.

It has been reported that the major monosaccharide composition of coconut meal was mannose (approximately 80%), followed by glucose, galactose, and a small amount of other sugars such as arabinose (Khuwijitjaru et al., 2012; Thongsook & Chaijamrus, 2014). Hence, mannose and mannooligosaccharides were used to represent mono- and oligosaccharides (DP1−DP6), respectively in this study. From the HPLC analysis, monosaccharides and mannooligosaccharides (DP2−DP6) were found in all hydrolysate samples (Table 1). The low to moderate treatment temperatures (120−200 °C) provided the hydrolysates with monosaccharides and mannooligosaccharides of 13.58−17.44 g/100 g hydrolysate, whereas the higher temperatures (210−230 °C) provided more mannooligosaccharides with a lower degree of polymerization. The treatment at 230 °C resulted in approximately 80 g/100 g hydrolysate of monosaccharides and mannooligosaccharides (DP1−DP6) and consisted of the high content of DP1−DP4 (74.71 g/100 g hydrolysate), while the high content of DP4−DP6 (22.38 g/100 g hydrolysate) could be obtained from the treatment at 220 °C.

As shown in Fig. 1D, 5-HMF was not found in the hydrolysates at lower treatment temperatures. 5-HMF contents of the hydrolysates from the treatments at 190−220 °C were detected to be only 0.02−0.44 g/100 g hydrolysate. However, 5-HMF content rapidly increased to 2.84 g/100 g hydrolysate at 230 °C. 5-HMF is an intermediate compound of the Maillard reaction and dehydration of sugars which is produced when the hexose loses three molecules of water in an acid-catalyzed system (Menegazzo et al., 2018). It is often found during subcritical water treatment of various raw materials (Wiboonsirikul et al., 2007). These results demonstrated that the subcritical water treatment promoted hydrolysis of carbohydrate components in defatted coconut meal, leading to the formation of oligomers, monomers, and other degradation products from monosaccharides such as furfural and 5-HMF as well as acid products.

The hydrolysate contained a small amount of protein (0−4.66 g/100 g hydrolysate). Initially, higher protein content could be obtained by increasing the temperature. The highest protein content was achieved from the subcritical water treatment at 160 and 180 °C ($p \leq 0.05$), and it significantly decreased at the higher treatment temperatures. Álvarez-Viñas et al. (2021) reported that the temperature close to 200 °C was the optimum for extracting protein from agricultural and food wastes and algae. However, Pińkowska et al. (2014) demonstrated that the proteins of rapeseed cake could be broken down into smaller peptides or amino acids at temperatures of 180−240 °C, and these components could be unmeasured by the Bradford assay (Chutipongtanate et al., 2012). These could be the cause of the lower or non-detectable protein concentrations in the hydrolysates at temperatures above 180 °C in this study, which also suggested that protein might be extensively hydrolyzed to small peptides and amino acids.

Precipitation of Carbohydrates

Since the subcritical water treatment resulted in the formation of undesired products such as monosaccharides and Maillard reaction products, the hydrolysate had to be purified before being used as a functional food ingredient. Figure 2A shows the yield of the precipitate obtained by precipitation in 90% v/v ethanol. The results show that the treatment temperature affected the precipitate yields, and the highest yield was obtained from the treatment at 180 °C. When considering the yield based on defatted coconut meal, an increasing temperature up to 220 °C gradually increased the precipitate yield. The highest yield

Table 1 Monosaccharide and mannooligosaccharide contents of the defatted coconut meal hydrolysate obtained by subcritical water treatment at various temperatures

Treatment temperature (°C)	Carbohydrate composition (g/100 g hydrolysate, d.b.)							
	Monosaccharides (DP1)	DP2	DP3	DP4	DP5	DP6	DP2 − 6	DP1 − 6
120	2.65 ± 0.08^{ab}	3.54 ± 0.10^{c}	3.21 ± 0.60^{c}	3.40 ± 0.59^{b}	2.91 ± 0.39^{b}	1.73 ± 0.15^{ab}	14.79 ± 1.57^{b}	17.44 ± 1.48^{b}
140	2.62 ± 0.05^{ab}	3.07 ± 0.04^{b}	2.50 ± 0.02^{b}	2.62 ± 0.03^{a}	2.63 ± 0.24^{ab}	1.95 ± 0.05^{ab}	12.76 ± 0.31^{a}	15.38 ± 0.35^{ab}
160	2.12 ± 0.12^{a}	2.27 ± 0.09^{a}	1.79 ± 0.11^{a}	2.67 ± 0.14^{ab}	3.00 ± 0.17^{c}	1.73 ± 0.16^{ab}	11.46 ± 0.33^{a}	13.58 ± 0.40^{a}
180	2.22 ± 0.12^{ab}	2.23 ± 0.12^{a}	2.41 ± 0.12^{b}	2.71 ± 0.19^{ab}	2.60 ± 0.21^{ab}	1.66 ± 0.07^{ab}	11.61 ± 0.56^{a}	13.83 ± 0.61^{a}
190	2.61 ± 0.10^{ab}	2.45 ± 0.19^{a}	2.68 ± 0.26^{bc}	2.68 ± 0.30^{ab}	2.28 ± 0.08^{a}	1.41 ± 0.03^{a}	11.51 ± 0.67^{a}	14.12 ± 0.77^{a}
200	2.82 ± 0.28^{ab}	2.17 ± 0.06^{a}	2.38 ± 0.17^{b}	2.75 ± 0.35^{ab}	2.85 ± 0.09^{b}	2.09 ± 0.07^{b}	12.25 ± 0.59^{a}	15.07 ± 0.45^{ab}
210	3.63 ± 0.10^{c}	3.91 ± 0.28^{c}	4.13 ± 0.17^{d}	4.15 ± 0.25^{c}	4.69 ± 0.44^{d}	3.20 ± 0.48^{c}	20.09 ± 1.55^{c}	23.71 ± 1.61^{c}
220	4.90 ± 0.07^{d}	6.13 ± 0.25^{d}	7.31 ± 0.61^{e}	7.47 ± 0.69^{d}	7.64 ± 0.41^{e}	7.28 ± 0.78^{d}	35.82 ± 1.88^{d}	40.72 ± 1.81^{d}
230	32.18 ± 0.96^{e}	21.92 ± 0.58^{e}	13.21 ± 0.26^{f}	7.41 ± 0.57^{d}	3.44 ± 0.25^{c}	1.94 ± 0.30^{ab}	47.91 ± 1.61^{e}	80.09 ± 2.55^{e}

Data are expressed as means ± standard deviation. Means in the same column with different lower-case superscripts are significantly different ($p \leq 0.05$, Duncan's multiple range test). DP2−6 is summation of DP2 to DP6. DP1−6 is summation of DP1 to DP6

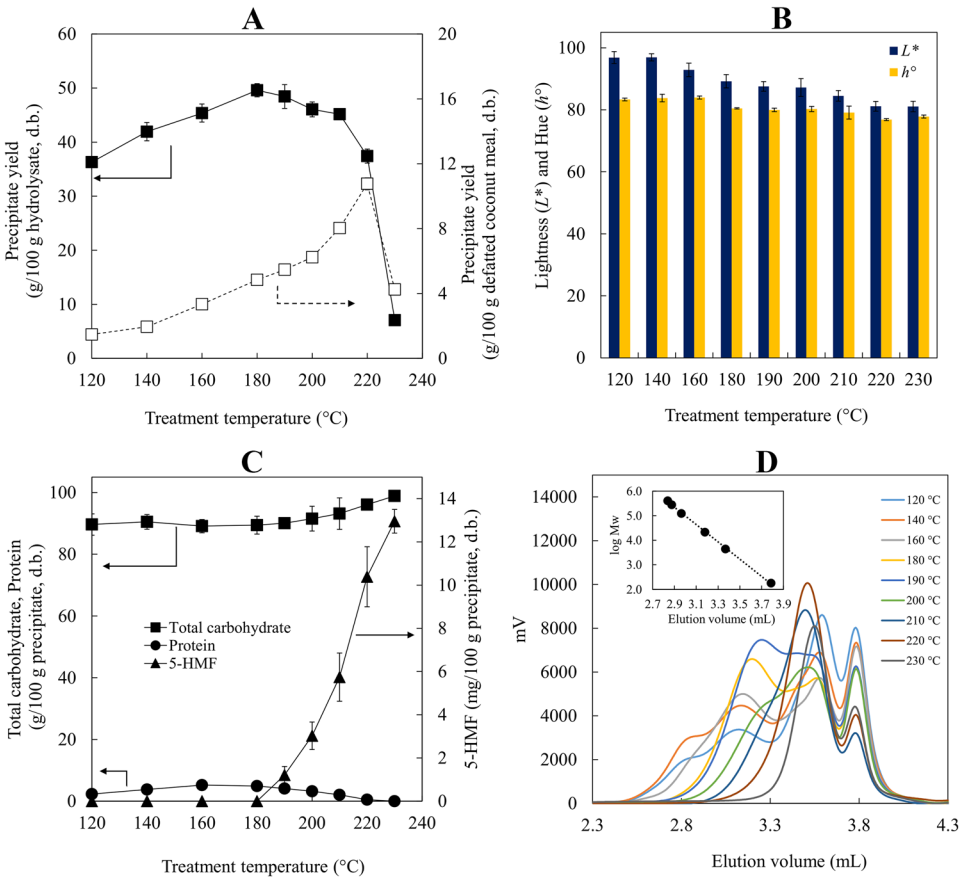

Fig. 2 Effect of subcritical water treatment temperature and ethanol precipitation on yields of precipitate (**A**), color of precipitates (**B**), chemical properties of precipitates (**C**), and size-exclusion chromatography of precipitates using refractive index detector (**D**)

was approximately 10.76%. However, the yield decreased rapidly to 4.25% at a treatment temperature of 230 °C. Precipitation using ethanol was an efficient method to separate carbohydrates from the hydrolysate because saccharides are polar; therefore, adding ethanol reduced the polarity of the system and carbohydrates' solubility, leading to their precipitation (Klinchongkon et al., 2019). Ethanol precipitation could also remove some colored impurities. The higher L^* and h values of dried precipitates indicated significant improvement in the color of the precipitates (Fig. 2B) compared to the hydrolysate (Fig. 1C). However, the colors of all precipitates were still in the range of yellowish to orange-yellow color. Figure 2C shows that the ethanol precipitation increased the total carbohydrates of the hydrolysate from approximately 74–91 to 86–99% with a maximum of 6% of protein. It should be noted that the phenol–sulfuric acid method may give slightly higher carbohydrate content values than the actual ones due to some interfering substances, e.g., furans from sugar degradation. Nevertheless, the 5-HMF content of the precipitates was significantly lowered. The highest 5-HMF value of 0.13 mg/g was found at 230 °C, which means that around 99.5% of 5-HMF in the precipitate (28.44 mg/g) was removed. The result of low amounts of remaining 5-HMF contents in the precipitates agreed with the improvement of color.

Mono- and oligosaccharides contents of the final products changed as shown in Table 2, and the HPLC chromatogram is shown in Supplementary Fig. S2. Monosaccharides in the precipitate decreased to 1.05–2.71 g/100 g. The short-chain mannooligosaccharides (DP2 and DP3) were also recovered in only a small amount, and the main compositions of the precipitates were DP5 and DP6. The results agreed with the previous study of Klinchongkon et al. (2019), indicating that DP2 and DP3 could not be effectively recovered by using 90% ethanol. Other purification techniques such as membrane filtration or size-exclusion chromatography may be able to improve their yields. However, considering the content of oligosaccharides from DP2 to DP6, the final product of the treatment at 230 °C showed the highest amount of these mannooligosaccharides, approximately 46% ($p \leq 0.05$), followed by the treatment of 220 °C (31.39%). While the products obtained from 120–210 °C contained only 7.98–13.11%, the lowest was from the treatment at 160 °C.

The M_w of the final products was significantly affected by the treatment temperature. The higher treatment temperatures resulted in lower M_w of precipitates (Table 2 and Fig. 2D) which indicated a higher degree of polysaccharide

Table 2 Monosaccharide and mannooligosaccharide contents and molecular weight of the precipitate produced from defatted coconut meal hydrolysate using ethanol precipitation with final ethanol concentrations of 90%

Treatment temperature (°C)	Carbohydrate composition (g/100 g precipitate, d.b.)								M_w
	Monosaccharides (DP1)	DP2	DP3	DP4	DP5	DP6	DP2–6	DP1–6	(kDa)
120	1.22 ± 0.11^{ab}	1.28 ± 0.19^{b}	1.68 ± 0.52^{b}	3.49 ± 0.52^{b}	3.97 ± 0.48^{a}	2.69 ± 0.34^{ab}	13.11 ± 1.08^{b}	14.33 ± 1.19^{c}	34.58 ± 1.19^{e}
140	1.05 ± 0.08^{a}	0.94 ± 0.02^{a}	0.76 ± 0.03^{a}	1.85 ± 0.14^{a}	2.67 ± 0.25^{a}	2.05 ± 0.21^{a}	8.27 ± 0.42^{a}	9.33 ± 0.34^{a}	34.70 ± 0.73^{e}
160	1.11 ± 0.06^{ab}	1.03 ± 0.09^{a}	0.93 ± 0.17^{a}	1.73 ± 0.26^{a}	2.30 ± 0.17^{a}	1.99 ± 0.15^{a}	7.98 ± 0.65^{a}	9.09 ± 0.59^{a}	11.79 ± 1.07^{d}
180	1.12 ± 0.03^{ab}	0.97 ± 0.14^{a}	0.77 ± 0.27^{a}	1.64 ± 0.53^{a}	2.43 ± 0.83^{a}	2.41 ± 0.50^{ab}	8.22 ± 2.17^{a}	9.34 ± 2.19^{a}	7.76 ± 0.51^{c}
190	1.30 ± 0.04^{b}	0.91 ± 0.06^{a}	0.90 ± 0.12^{a}	1.67 ± 0.23^{a}	2.45 ± 0.38^{a}	2.49 ± 0.24^{ab}	8.43 ± 0.76^{a}	9.73 ± 0.77^{ab}	5.50 ± 0.32^{b}
200	1.16 ± 0.14^{ab}	1.01 ± 0.09^{a}	1.14 ± 0.36^{ab}	1.98 ± 0.50^{a}	3.45 ± 0.88^{a}	3.80 ± 0.67^{bc}	11.39 ± 2.35^{ab}	12.56 ± 1.99^{bc}	1.81 ± 0.17^{a}
210	1.11 ± 0.09^{ab}	0.97 ± 0.12^{a}	0.99 ± 0.13^{a}	2.09 ± 0.20^{a}	4.01 ± 0.54^{a}	4.84 ± 0.61^{c}	12.90 ± 0.73^{b}	14.02 ± 0.64^{bc}	1.22 ± 0.07^{a}
220	1.18 ± 0.14^{ab}	1.48 ± 0.25^{b}	2.65 ± 0.64^{c}	5.79 ± 1.48^{c}	10.57 ± 2.17^{b}	10.91 ± 1.01^{d}	31.39 ± 5.12^{c}	32.57 ± 5.11^{d}	1.13 ± 0.06^{a}
230	2.71 ± 0.17^{c}	2.65 ± 0.20^{c}	2.93 ± 0.37^{c}	6.53 ± 0.59^{c}	16.32 ± 1.14^{c}	17.69 ± 1.81^{e}	46.12 ± 3.34^{d}	48.83 ± 3.37^{e}	0.83 ± 0.03^{a}
Inulin	-	-	-	-	-	-	-	-	1.56

Data are expressed as means ± standard deviation. Means in the same column with different lower-case superscripts are significantly different ($p \leq 0.05$, Duncan's multiple range test). DP2–6 is summation of DP2 to DP6. DP1–6 is summation of DP1 to DP6. The weight-average molecular weight (M_w) was calculated from size-exclusion chromatography as shown in Fig. 2D

hydrolysis. The highest M_w was found from treatments at 120 °C and 140 °C. Their chromatograms showed four peaks with various elution volumes, implying that these precipitates contained low to high molecular weight saccharides. When the treatment temperature was raised to 160 °C and above, the first peak shifted from 2.91 to 3.12–3.55 mL elution volume, and the M_w was significantly lowered ($p \leq 0.05$). The final products obtained at 210–230 °C were a mixture of small saccharides and oligosaccharides since the chromatograms showed the first peak at 3.50–3.55 mL and the second peak at 3.78 mL elution volume. The M_w below 1 kDa was achieved from the subcritical water treatment at 230 °C. However, it was not significantly different from the M_w of the treatment at 200–220 °C ($p > 0.05$). These results agreed with previous studies which reported that subcritical water treatment was an efficient approach for the hydrolysis of different polysaccharides, e.g., pumpkin polysaccharides (Yu et al., 2021), galactomannan in the spent coffee ground (Getachew et al., 2018), cellulose in sesame hull (Zhang et al., 2021), and pectin in apple pomace (Zhang et al., 2022). The chromatograms in Fig. 2D also suggested that there were high molecular weight carbohydrates in the precipitates.

Antioxidant Activity

DPPH radical scavenging activity and FRAP results are shown in Fig. 3. Both DPPH radical scavenging activity and FRAP increased with the temperature ($p \leq 0.05$). Their highest values were found with the precipitate obtained at 230 °C (8.25 and 33.91 mmol TE/g precipitate, respectively). FRAP assay presented higher values for every treatment, implying the good reducing ability of the compounds in the precipitates. The antioxidant activity of the precipitates may be from phenolic compounds, proteins, and products from various reactions since different compounds could be extracted by the subcritical water treatment. Plaza et al. (2010) demonstrated that the overall antioxidant capacity of the extracts was affected by compounds obtained from the Maillard reaction, caramelization, and thermoxidation reaction during subcritical water extraction, whereas, Narita and Inouye (2012) reported a high correlation of antioxidant activity with protein and phenolic content in the coffee silverskin extracts obtained by subcritical water extraction.

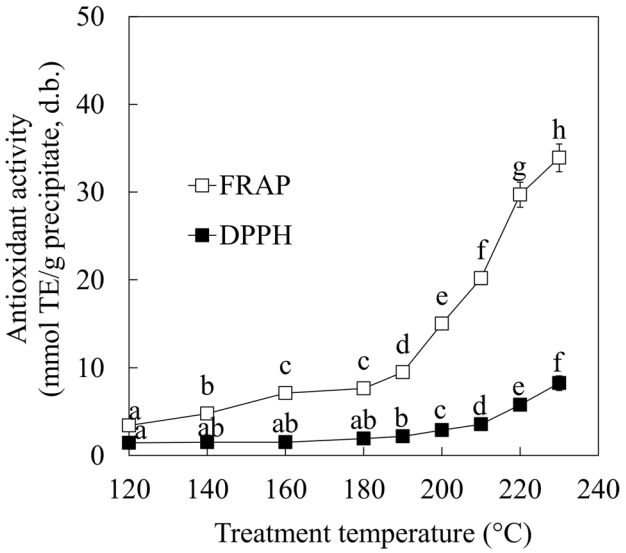

Fig. 3 Antioxidant activity of precipitates. Different letters indicate significant differences among treatments ($p \leq 0.05$, Duncan's multiple range test)

In Vitro Fermentation

Figure 4 shows the prebiotic property of the mannooligosaccharide-rich carbohydrates evaluated by in vitro fermentation for 48 h expressed as growth (ΔA_{600}) of *L. rhamnosus* (TISTR 2443), *L. plantarum* (TISTR 1265), *B. bifidum* (TISTR 2129), and *B. breve* (TISTR 2130), compared to the control medium (without carbohydrate source), glucose, and inulin. It was found that all mannooligosaccharide-rich carbohydrates from the subcritical water treatment resulted in similar growth to that of inulin for *L. plantarum* (TISTR 1265), *L. rhamnosus* (TISTR 2443), and *B. bifidum* (TISTR 2129), but slightly lower for *B. breve* (TISTR 2130). In comparison to the control, most mannooligosaccharide-rich carbohydrates resulted in higher growth of *L. rhamnosus* (TISTR 2443) ($p \leq 0.05$) except the treatment at 140, 160, 180, and 190 °C, which contained the lower content of DP2−DP6 than other treatment temperatures (Table 2). Similarly, the growth of *L. plantarum* (TISTR 1265) and *B. bifidum* (TISTR 2129) could be significantly increased with the mannooligosaccharide-rich carbohydrates from 220 and 230 °C while *B. breve* (TISTR 2130) was enhanced by the mannooligosaccharide-rich carbohydrates only at 230 °C. The growth of all probiotics with inulin and mannooligosaccharide-rich carbohydrates was only marginally higher than the control. This might be ascribed to the relatively low amount of carbohydrate sources used in this study (0.2% w/v) due to the limitation of sample availability. These results indicated that mannooligosaccharide-rich carbohydrates produced by subcritical water treatment could enhance the growth of *Lactobacillus* spp. and *Bifidobacterium* spp. similar to mannooligosaccharides derived from copra meal using mannanase and mannooligosaccharides obtained from other plant mannans such as konjac, guar gum, locust bean gum, and palm kernel meal (Jana et al., 2021).

The media containing mannooligosaccharide-rich carbohydrate obtained at 230 °C were analyzed by thin-layer chromatography (TLC) to confirm the utilization of oligosaccharides by each bacterial strain during the fermentation. Briefly, the media were loaded on a silica gel–coated aluminum plate (TLC Silica gel 60 F_{254}, 20 cm × 20 cm, Merck, Darmstadt, Germany). The solvent system was a mixture of ethyl acetate/acetic acid/water at the ratio 2:2:1 by volume (Kumar et al., 2018). The visualizing solution was prepared from ethanol and sulfuric acid (95:5 v/v) and the TLC plate was heated at 120 °C for 10 min. As shown in Fig. 5, after

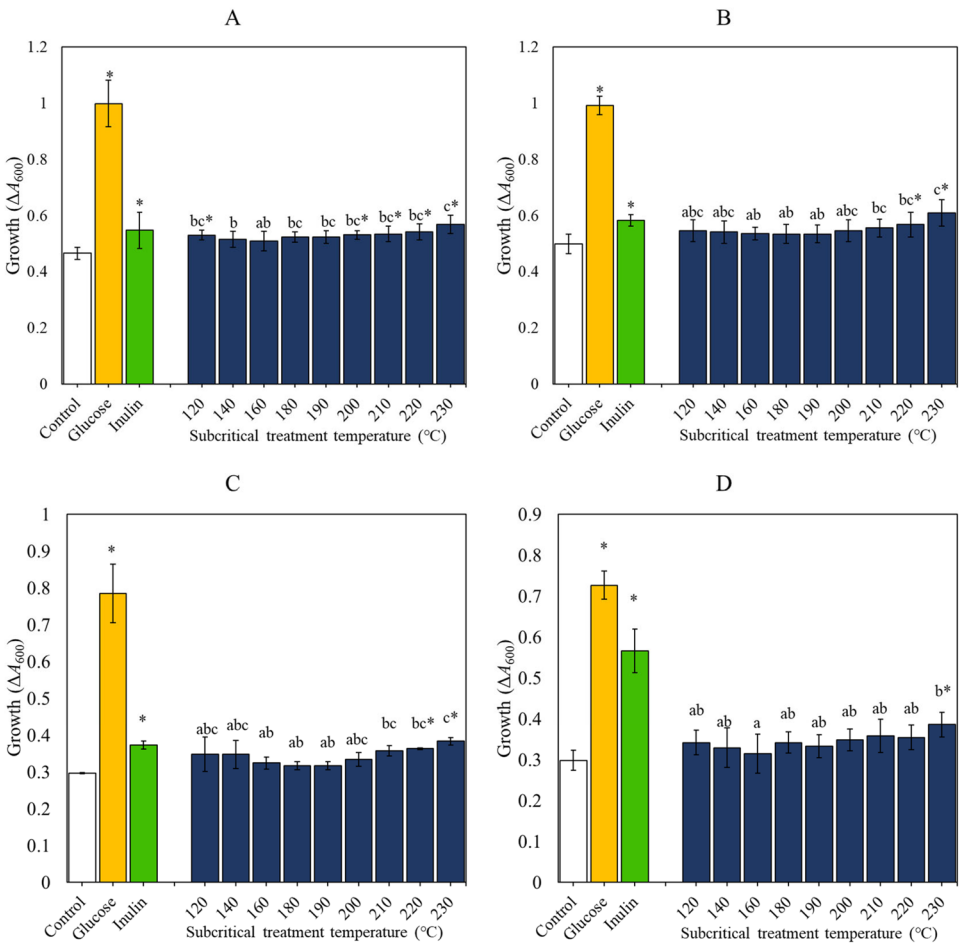

Fig. 4 Growth (ΔA_{600}) of *L. rhamnosus* (TISTR 2443) (**A**), *L. plantarum* (TISTR 1265) (**B**), *B. bifidum* (TISTR 2129) (**C**), and *B. breve* (TISTR 2130) (**D**) in media containing mannooligosaccharide-rich carbohydrates obtained at various temperatures (120–230 °C), media with glucose (Glucose), and media with inulin (Inulin), compared with the growths in media without carbon (Control). Different letters indicate significant differences in the subcritical treatments ($p \leq 0.05$, Duncan's multiple range test) and asterisk (*) indicates significant differences from Control ($p \leq 0.05$, Dunnett's test)

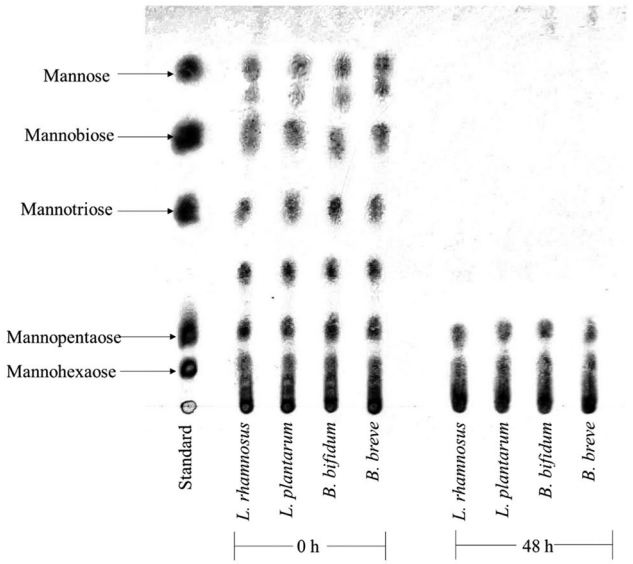

Fig. 5 Thin-layer chromatography of media containing mannooligosaccharide-rich carbohydrate obtained at 230 °C before (0 h) and after fermentation with *L. rhamnosus* (TISTR 2443), *L. plantarum* (TISTR 1265), *B. bifidum* (TISTR 2129), and *B. breve* (TISTR 2130) for 48 h

incubation for 48 h, monosaccharides and DP2−DP4 oligosaccharides were not found in the media, while DP5 also showed less intensity, indicating that all the probiotic strains utilized these small saccharides during the fermentation. Manning and Gibson (2004) suggested that the growth of probiotics can be affected by the monosaccharide composition, glycosidic linkage type, and the degree of polymerization. These results suggested that the coconut meal oligosaccharides with high DP (DP > 5) seemed not to stimulate the growth of the strains. Kalidas et al. (2017) reported that low-DP mannoologisaccharides generated from palm kernel cake promoted the growth of lactobacilli, while DP > 5 did not support the growth of *L. plantarum, L. casei ver. Rhamnosus,* and *L. acidophilus*. A similar result has been found for inulin. Biedrzycka and Bielecka (2004) demonstrated that short-chain fructooligosaccharide and oligofructose as well as short-chain inulin could be utilized by bifidobacteria and promoted its growth, whereas mildly polymerized (DP ≥ 9) and highly polymerized (DP ≥ 22) inulins did not significantly affect the growths of bifidobacteria.

Short-Chain Fatty Acids and Lactic Acid

Short-chain fatty acids are the main product of colonic bacteria fermentation on non-digestible oligosaccharides (Louis et al., 2014). After 48 h of incubation, the results from HPLC analysis showed that the short-chain fatty acid and lactic acid concentrations in the media with oligosaccharides obtained at different treatment temperatures were not significantly different ($p > 0.05$), and these values were similar to that of inulin.

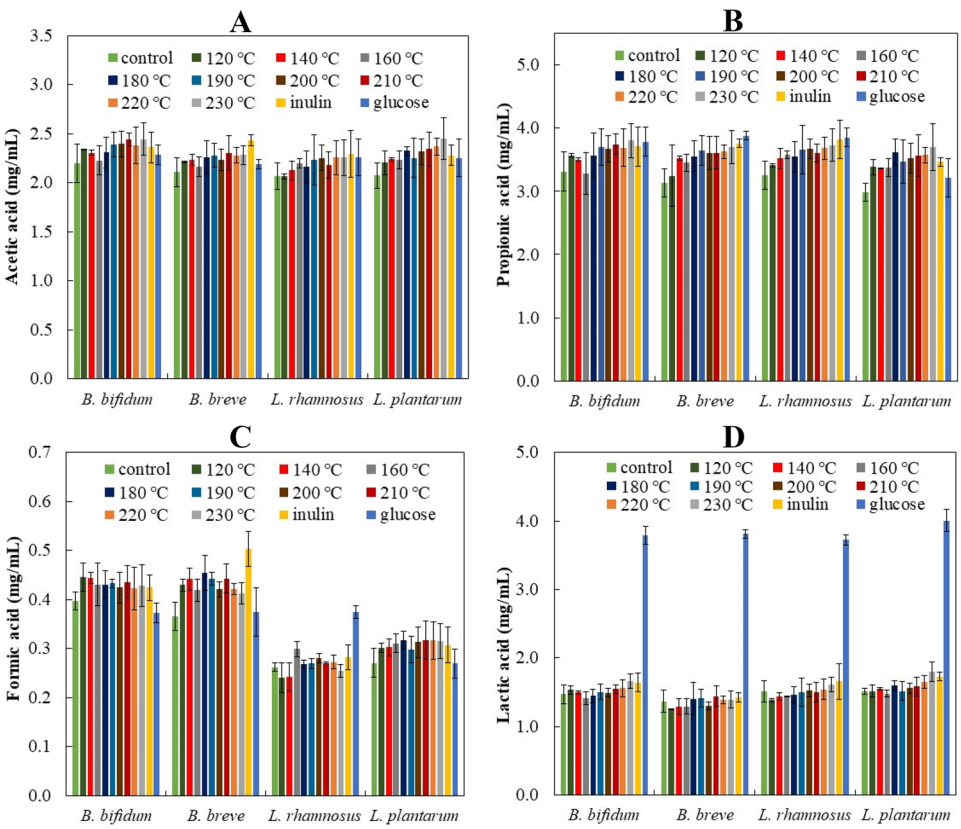

Fig. 6 Short-chain fatty acids and lactic acid production by *L. rhamnosus* (TISTR 2443), *L. plantarum* (TISTR 1265), *B. bifidum* (TISTR 2129), and *B. breve* (TISTR 2130) after 48 h fermentation without carbon source (control) and with mannooligosaccharide-rich carbohydrates from subcritical water treatment of coconut meal at 120−230 °C, inulin, and glucose: acetic acid (**A**), propionic acid (**B**), formic acid (**C**), and lactic acid (**D**)

The chromatogram is shown in Supplementary Fig. S3. The major short-chain fatty acids were acetic acid (2.06−2.45 mg/mL) (Fig. 6A) and propionic acid (3.24−3.80 mg/mL) (Fig. 6B), while formic acid (Fig. 6C) was found in a small amount (0.24−0.45 mg/mL). The results agreed with previous studies (Suryawanshi & Kango, 2021; Shubhashini et al., 2022), which found that the main products of copra meal and defatted-copra meal mannooligosaccharides fermented by lactobacilli were acetic and propionic acids. However, in this study, the fermented medium did not contain butyric acid, even with the inulin. The production of these short-chain fatty acids and lactic acid resulted in a lowering of the pH of the fermented medium from 6.80 to 5.38−6.78, depending on their concentrations. Short-chain fatty acids compositions and concentrations were influenced by the type and content of the substrate (Ashaolu et al., 2019; Neri-Numa & Pastore, 2020). While glucomannan increased the production of acetic and propionic acids (Jonathan et al., 2012), other oligosaccharides, i.e., fructooligosaccharides, cellulose, and pectin, have been demonstrated to improve acetic and butyric acid production (Jonathan et al., 2012; Li et al., 2015; Yang et al., 2013). Short-chain fatty acids play key roles in the host's health. Fermentation of the mannooligosaccharide-rich carbohydrate provided propionic acid which has shown anti-cancer properties and the ability to boost the metabolism of lipids and inhibit liver cholesterol synthesis (Ashaolu et al., 2021). Mannooligosaccharide consumption could reduce adipose tissue, serum lipids, body fat, and body weight gain in addition to reducing insulin resistance (Jana et al., 2021).

Conclusions

This study demonstrated that subcritical ethanol and subcritical water treatments could be applied for producing mannooligosaccharide-rich carbohydrates from coconut meals. The obtained carbohydrates exhibited antioxidant properties and could be metabolized by probiotics. The obtained carbohydrates also promoted the growth of *L. plantarum* (TISTR 1265), *L. rhamnosus* (TISTR 2443), *B. bifidum* (TISTR 2129), and *B. breve* (TISTR 2130) and production of short-chain fatty acids similar to commercial inulin. Thus, the mannooligosaccharide-rich carbohydrates obtained from defatted coconut meals using subcritical fluid treatment may be potentially applied as a functional food ingredient.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11947-022-02954-6.

Author Contribution T. P. wrote the main manuscript text and prepared all tables and figures. T. P. and P. K. designed the experiment. P. K., K. K., and S. A. supervised the study. P. K. and K. K. acquired the funding. All authors reviewed the manuscript.

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Availability of Data and Material Research data are available from the corresponding author upon request.

Code Availability Not applicable.

Declarations

Conflict of Interest The authors declare no competing interests.

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