

# Partial Characterization of Polysaccharides Isolated from Defatted Desiccated Coconut Kernel

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## Abstract

Industrial processing of coconut oil generates considerable biomass of dehydrated defatted coconut kernel (DDCK) which is composed of food-grade fiber. The objective of this study was to separate and isolate polysaccharides and oligosaccharides in defatted coconut kernel, and to determine the partial structure using sugar profile. Coconut cell wall polysaccharides (CCWP) obtained from DDCK were used to sequentially extract pectin (with 5% ammonium oxalate-oxalic acid), HCl (with 4% NaOH), HCII (with 10% NaOH), HCIII (with 17.5% NaOH) and the remaining fraction as non-extractible matter referred as NaOH non-extractible. Results indicated that CCWP composed of 19% pectin, 29.6% HCl, 12% HCII, no detectable HCIII and NaOH non-extractible of 15%. The sugar profile of pectin, was 6.14% rhamnose, 3.31% arabinose, 61.72% mannose and 18.71% galactose. HCl composed of rhamnose 13.29%, arabinose 4.49%, xylose 22.84%, mannose 50.98%, galactose 5.9% and glucose 3.39%. HCII contained rhamnose 37.12%, arabinose 3.35%, Mannose 27.15% and galactose 5.76%, while NaOH non-extractible contained rhamnose 22.5%, mannose 23.95%, galactose 16.35% and glucose 37.05%. Partial hydrolysis followed by concentration with Sephadex G15 size exclusion chromatography was able to separate oligosaccharide having rhamnose 15.3%, mannose 52.5% and galactose 32.2% from pectin while xylose 0.6%, mannose 95.6%, galactose 1.1% and glucose 2.7% from HCII. Results indicated the presence of rhamnogalactomannan and xylogalactoglucoman in coconut kernel.

Keywords: Coconut kernel, pectin, hemicellulose, cellulose

## Introduction

Coconut (*Cocos nucifera* L.) is a multipurpose crop that covers 12.5 million hectares of land in the world (Coconut Statistics, 2021). World coconut oil production is around 3.51-3.63 million metric tons ([www.statista.com](http://www.statista.com), 2022). Therefore, world coconut oil processing produces approximately 2.4 million metric tons of coconut meal. Part of copra meal is used as animal feed; however, it can be used to extract cellulose, hemicellulose and pectin for food and industrial use.

Cell wall polysaccharides are a combination of pectin, hemicellulose, and cellulose. Plant-based foods are rich in those chemical components. During processing plant materials for food purposes, most of the polysaccharides are removed. As an example, edible oil extraction, fruit juice extraction and cereal processing lots of byproducts are generated as agricultural biomass, which are rich in pectin, hemicellulose and cellulose

which are considered food-grade fiber (Audenhove, 2021; Olmos and Hansen, 2012; Government et al., 2019).

Many agricultural wastes have been researched for the extraction of carbohydrate and oligosaccharide extraction. Sugar beet pulp (Olmos and Hansen, 2012; Knež et al., 2018), wheat straw (Huang et al., 2021), tomato (Audenhove, 2021), sorghum stem (Xu et al., 2018) and mango seed shell (Government et al., 2019) are some of such studies. The use of coconut kernel residue, which is left after oil extraction from coconut kernel, has not been studied for the separation of polysaccharides and oligosaccharides. So, this study focused on studying the separation of carbohydrate fractions and oligosaccharides which have the potential for use in the food industry. Polysaccharides and oligosaccharides are used as dietary fiber in food industry. They are popular as thickening, emulsifying and stabilizing agents and are used for improving the texture of food and beverages. They are also used as fat replacers, prebiotics, and antiulcer agents in the food industry.

Few studies have been reported on the composition of coconut kernel polysaccharides. Srirakul and Keawsompong (2021) proposed alkaline extraction of copra meal as a way of extracting polysaccharides for use in pharmaceuticals, biomedical and feed and food industries. Yalagama et al. (2022) isolated cell wall polysaccharides such as insoluble, water-soluble, and neutral detergent soluble, neutral detergent insoluble, acid detergent soluble and acid detergent insoluble. Nor et al. (2017) reported that water-soluble crude polysaccharide had prebiotic characteristics and could stimulate the proliferation of *Lactobacillus casei* Shirota and *Lactobacillus bulgaricus*. Hemicellulose can be used to prepare oligosaccharides such as galactoglucomanan, which has prebiotic properties. Hemicellulose and oligosaccharides derived from plants are reported to have shown prebiotic properties (Polari et al., 2012). Therefore, polysaccharides are important as a functional food ingredient.

Hemicellulose is a relatively low molecular weight polysaccharide associated with cellulose and lignin through hydrogen bonds and various covalent bonds in the plant cell wall. Several chemical and biological methods are available for the extraction of hemicelluloses and cellulose. Ji et al. (2018) concentrated alkali-soluble oligosaccharides from ginseng roots using 50 mM Na<sub>2</sub>CO<sub>3</sub>, 1M KOH & 4M KOH followed by treating sequentially with hot water,  $\alpha$ -amylase and EDTA extraction. Ataei et al. (2020) used both high and low concentrations of alkali and commercially available xylanases to isolate xylooligosaccharides from date seeds. With the change of extraction solution, the structure of polysaccharide is also changed (Price et al., 2018). Sequential alkaline extraction is one of the most effective methods used for the isolation of hemicellulose (Fu et al., 2018).

As stated by Nor et al. (2017) and Polari et al. (2012), polysaccharides have pre-biotic properties and can be used in the production of functional foods. They can be used as an ingredient in products with high dietary fiber. The health benefits of dietary fiber are improvement of gut microflora, improvement of colonic health, prevention of constipation, maintenance of favorable body weight and reduced cardiovascular disease (Barber et al., 2020). This study aims to separate and isolate polysaccharides and oligosaccharides in defatted coconut kernel, and to determine the partial structure using a sugar profile.

## Material and Methods

### Material

Dehydrated desiccated coconut was obtained from virgin coconut oil production. Cell wall polysaccharides of coconut kernels (CCWP) were extracted using a method reported by Yalagama et al. (2022).

All the chemicals used in this study were analytical grade unless otherwise specified.

### Methods

#### *Sequential extraction of polysaccharides in coconut kernel cell wall polysaccharides*

Coconut kernel cell wall polysaccharides (CCWP) were separated into pectin, hemicellulose and cellulose using the method reported by Del Rosario et al. (1980) with modifications.

#### *Separation of pectin*

Five grams of CCWP were added to 100 ml of 0.5% ammonium oxalate-oxalic acid (prepared by dissolving 0.5 g ammonium oxalate and 0.5 g oxalic acid in 100 ml water). The extraction was carried out for 24 hours with stirring at 90°C under reflux. The supernatant was separated by centrifugation (Centurion Scientific Limited, UK; 5000 rpm) and the solid part was re-extracted for 24 hours using the same procedure. The supernatants were combined and 4 volumes of ethanol (96%) were added slowly with gentle stirring. The pectin was precipitated out by standing at 4°C overnight. The precipitates were collected by centrifugation at 5000 rpm for 30 minutes. The pellet was freeze-dried (CHAIST, Martin Christ, Germany) to a constant weight.

#### *Separation of hemicellulose*

##### *Isolation of Hemicellulose fraction I*

The residue obtained after the separation of pectin was placed in a conical flask and 400 ml of 4% NaOH was added. Liquid paraffin (½ cm thickness) was added and the contents were shaken for 18 hours at room temperature. The supernatant was separated from the insoluble matter by centrifugation at 11,000 rpm and was neutralized with hydrochloric acid. Four volumes of alcohol (96%) were added to the neutralized supernatant and left overnight to precipitate hemicellulose fraction I (HCI). The precipitate was separated by centrifugation at 11,000 rpm and was washed with alcohol and acetone. The precipitate was freeze-dried to a constant weight.

##### *Isolation of Hemicellulose fraction II*

The residue left after the separation of HCI was extracted with 400 ml of 10% NaOH. The surface was covered with liquid paraffin as mentioned previously. The contents were shaken for 18 hours. The NaOH soluble matter was separated from the insoluble matter by centrifugation. The insoluble portion was re-extracted with 10% NaOH, and the insoluble and soluble parts were separated as mentioned above. The soluble parts were combined and then neutralized with HCl. Four volumes of alcohol were added to the neutralized supernatant and left overnight at 4°C for precipitation of Hemicellulose fraction II (HCII). The precipitate was separated by centrifugation at 11,000 rpm. Finally, it was washed with ethanol and acetone. The precipitate was freeze-dried to a constant weight and the percentage yield was recorded.

##### *Isolation of Hemicellulose fraction III*

The same procedure as in the isolation of HCII was followed using 400 ml of 17.5% NaOH instead of 10% NaOH to isolate HCIII from the residue left after 10% NaOH.

#### *Isolation of NaOH non-extractive*

The residues left after the separations of hemicellulose and pectin are considered as the part that is resistant to digestion and hence referred to as NaOH non-extractive.

The extraction procedure was carried out in triplicate.

#### *Neutral sugar compositions in polysaccharide fractions*

##### *Derivatization of alditol acetates of neutral sugars in cell wall polysaccharides*

The polysaccharides (pectin, HCl, HClI and NaOH non-extractible matter) were hydrolyzed separately in 2M TFA (0.1 g of sample in 10 ml of acid) at 100°C for 14-18 hours. The hydrolysate was filtered through the Whatman 42 filter paper. Excess acid in the filtrate was removed by vacuum evaporation and co-evaporation with water to remove all the traces of acids. The hydrolysate containing monosaccharides was converted to alditol acetates using the methods reported by Blakeney et al. (1983). The standard monosaccharides, glucose, arabinose, xylose, mannose galactose and rhamnose of analytical grade were also converted to alditol acetates using methods reported by Blakeney et al. (1983).

##### *Analysis of alditol acetates and determination of sugar profile*

Alditol acetates were analyzed using Gas Chromatograph (Agilent 4890D, Agilent Technologies (Pvt) Ltd., USA), Column – DB 23, 30 m x 0.32 mm x 0.25 µm film thickness, Injection volume – 1.0 µl, Detector – FID, Program – Set temperature – 200°C, Initial time - 40 min, Final temperature – 200°C, run time – 40 minutes, Injector temperature - 275°C, Detector temperature - 260°C. Sugar alditol acetates were dissolved in chloroform and 1 µl was injected into the GC. Individual alditol acetates of standard sugars were injected separately and the retention times were identified. Then the samples were injected and the peaks were identified. The total area under each monosaccharide peak was calculated. Each analysis was carried out in triplicate. The mean values of each monosaccharide were calculated.

#### ***Partial hydrolysis of Pectin and Hemicellulose II***

The partial hydrolysis was carried out with 0.2M TFA. Dilute TFA (0.2M TFA, 25 ml) was added to 0.1 g of each sample. The contents were boiled under reflux for 5 hours. The contents were concentrated under a vacuum and the excess acid was removed with co-evaporation with water. The partially hydrolyzed samples were separated in the Sephadex G 15 column. The fractions relevant to peaks were combined and freeze-dried.

#### ***Determination of the oligosaccharides in partially hydrolyzed polysaccharides***

The oligosaccharides in partially hydrolyzates obtained from pectin and HClI were determined with separation

by Sephadex G15 followed by neutral sugar profile of the respective oligosaccharides using the following method.

#### *Column preparation*

Sephadex G – 15 (10 g) was made into a slurry with distilled water and was washed several times to remove soluble sugars. The supernatant was tested for the presence of sugar using a phenol sulphuric reagent (Dubios, 1956). The glass column was filled with the slurry up to 40 cm and the eluent was tested for sugar, the confirmation of the absence of sugars in the eluents. The column was ready for use when the eluent was negative for phenol sulphuric acid reagent.

The inclusion volume of the column was determined by loading the column with 100 ppm glucose. The glucose was eluted with distilled water until it was completely eluted. Five ml fractions of eluent were collected. Each fraction was detected for the presence of glucose using a phenol sulphuric acid reagent. The elution profile was obtained by plotting absorbance vs elution volume.

#### *Separation of oligosaccharides in partially hydrolyzed polysaccharides*

After the determination of the inclusion volume of the column, the sample (partially hydrolyzed pectin) was placed on the top of the column. The sample was dissolved in water (0.2 g / 5 ml water) and filtered through the Whatman 42 filter paper. The sample was eluted with distilled water at a flow rate of 0.5 ml/minute. The eluent of 5ml fractions was collected. The sugar content of each fraction was observed using phenol sulphuric acid reagent (Dubios, 1956). The elution profile was obtained by plotting absorbance vs fraction volume. The fractions relevant to each peak were combined and freeze-dried to obtain oligosaccharide from pectin. The procedure was repeated for partially hydrolyzed HClI to isolate oligosaccharides from HClI.

The sugar profile of the oligosaccharide fractions obtained from pectin and HClI were determined by hydrolyzing, reduction and acetylation, as described above for determination of the partial structure of oligosaccharides.

## **Results and Discussion**

### **Results**

#### *Sequential extraction of hemicellulose and cellulose from Insoluble polysaccharides of coconut kernel*

The Cell wall polysaccharides of coconut kernel (CCWP) were fractionated into several polysaccharide fractions (Figure 1) and the yield of percentages of the fractions are given in Table 1.

Table 1 shows that the major fraction of CCWP was hemicellulose I (HClI) (29.6%) followed by pectin, hemicellulose II (HClI) and cellulose. Hemicellulose

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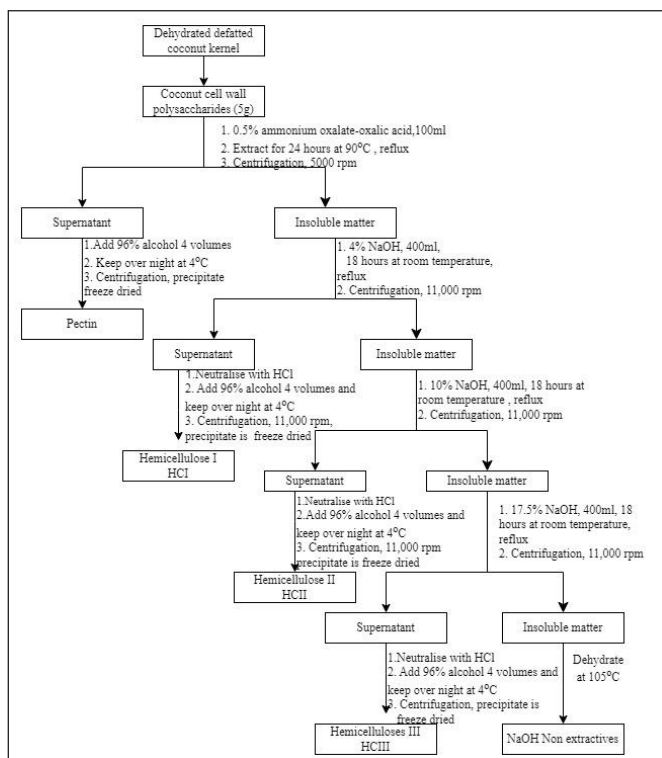


Figure 1. Separation of hemicellulose from defatted desiccated coconut kernel

Table 1. Percentage yield of polysaccharides separated from sequential extraction of Cell wall polysaccharide of coconut kernel using Sodium hydroxide

Extraction	Fraction	Amount
0.5% ammonium oxalate – oxalic acid	Pectin	19.0 %
4 % NaOH	Hemicellulose I	29.6 %
10 % NaOH	Hemicellulose II	12.0 %
17.5 % NaOH	Hemicellulose III	Trace
Non-extractable matter	NaOH non-extractive	15.0 %
Total cell wall polysaccharides		75.6 %

III (HCIII) was in trace amounts. The total cell wall polysaccharides isolated using sequential extraction with increasing concentration of NaOH is 75.6%. The proximate composition of CCWP shows that it contained crude fiber and carbohydrates of 34.0% and 44.13%, respectively (Yalegama et al., 2022). Therefore, the sequential extraction using NaOH has extracted almost all the crude fiber and carbohydrates (~97%) available in CCWP.

### Neutral sugar composition of sequentially extracted fractions

The fractions pectin, HC I and HC II were separately hydrolyzed using 2M TFA for 14 hours and cellulose (NaOH insoluble) fraction was hydrolyzed for 18 hours. The sugar profile of each fraction is given in Table 2.

Table 2. Sugar profile of polysaccharides of coconut cell wall polysaccharides

Neutral sugar %	Fraction			
	Pectin	HCI	HCII	NaOH non-extractive
Rhamnose	6.14 %	13.29 %	37.12 %	22.5 %
Arabinose	3.31 %	4.49 %	3.35 %	ND
Xylose	ND	22.89 %	ND	ND
Mannose	61.72 %	50.98 %	27.15 %	23.95 %
galactose	28.71 %	5.90 %	5.76 %	16.35 %
glucose	ND	3.34 %	ND	37.05 %

ND – not detected

Table 3. Percentage yield of oligosaccharides obtained from partially hydrolyzed pectin and hemicellulose II

Fraction	Yield (g)	Percentage yield
Partially hydrolyzed pectin	0.053	53%
Partially hydrolyzed HCII	0.066	66%

Table 2 shows that the pectin fraction separated from CCWP contains rhamnose 6.14%, arabinose 3.31%, mannose 61.72% and galactose 28.71%. The pectin fraction does not contain glucose and xylose in detectable levels. The ratio of rhamnose:arabinose:mannose:galactose is 9:5:93:43. The high ratio of mannose and galactose indicates that the pectic polysaccharide chain consists of galactomannan residues or arabinorhamnogalactomannan.

The solubilization of CCWP with 4% NaOH resulted in the separation of hemicellulose (HCI), which contains mannose (50.98%), followed by 22.89% xylose, 13.29% rhamnose, 5.90% galactose, 4.49% arabinose and glucose 3.34%. The ratio of rhamnose:arabinose:xylose:mannose:galactose:glucose of HCI is 4:1:7:15:1. HCI contains almost all the neutral sugars and is a complex hemicellulose. The sugar profile indicated that HCI is a mannan-based hemicellulose.

When the concentration of NaOH increased from 4% to 10%, a polysaccharide having rhamnose as a major neutral sugar was separated (Table 2). HCII contains only 4 neutral sugars, which are rhamnose, arabinose mannose and galactose. The mole ratio is rhamnose:arabinose:mannose:galactose is 11:1:8:2. The results indicate that HCII is a rhamnan-based hemicellulose.

The NaOH non-extractive contains different neutral sugars. It mainly contains glucose 37.05%; in addition to that galactose 16.35%, mannose 23.95% and rhamnose 22.5%. The presence of other monosaccharides indicated the incomplete fractionation in a lower concentration of NaOH and was referred to as NaOH non-extractive.



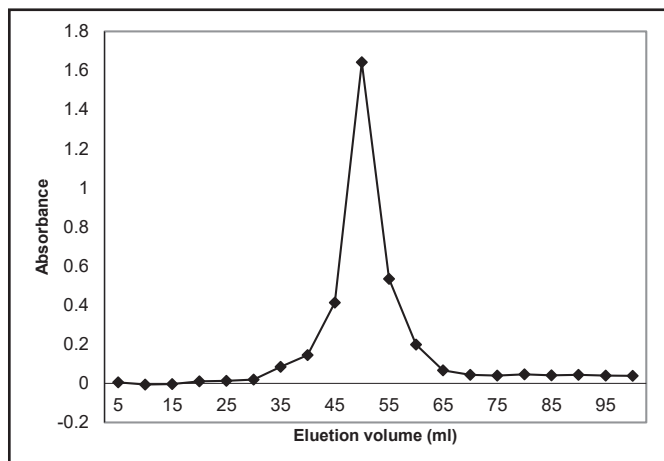


Figure 2. Elution profile of partially hydrolyzed pectin fraction on Sephadex G-15

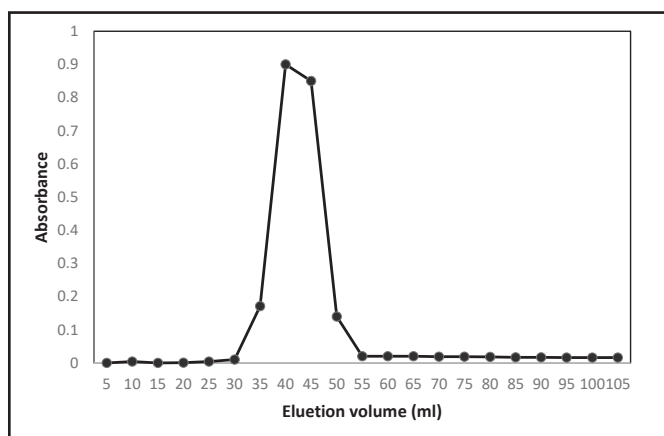


Figure 3. Elution profile of partially hydrolyzed HCII on Sephadex G-15

**Characterization of sequentially fractionated polysaccharides of CCWP**

Sequentially fractionated polysaccharides pectin and HC II were partially hydrolyzed to concentrate oligosaccharides using 0.2M trifluoro acetic acids. The concentrated partially hydrolyzed solutions were separated on the Sephadex G15 column with distilled water as the eluent. Figures 2 and 3 show the relevant elution profiles.

The yield of oligosaccharide fractions obtained from partially hydrolyzed pectin and HCII is shown in Table 3. As a result of partial hydrolyzation of pectin substances of CCWP, 53% of oligosaccharides were obtained. Similarly, partially hydrolyzation of HCII produced 66% of oligosaccharides.

The partially hydrolyzed pectin showed one single peak when separated with a Sephadex G15 column. Sephadex G15 separate dextran having molecular weights greater than 1500 KD (<https://www.sigmaaldrich.com/LK/en/product/sigma/g15120>). The fractions from 7-13 (35-65 ml) were combined and freeze-dried (Figure 2). The elution profile in Figure 2 shows only one peak, indicating that the partially hydrolyzed pectin fraction may contain only one polysaccharide.

Table 4. Sugar profile of partially hydrolyzed pectin and HCII

Neutral sugar	Partially hydrolyzed pectin	Partially hydrolyzed HCII
Rhamnose	15.3	ND
Arabinose	trace	ND
Xylose	ND	0.6
Mannose	52.5	95.6
Galactose	32.2	1.1
Glucose	ND	2.7

ND- not detected

The partial hydrolysate of HCII shows only one peak at fractions 7-12 (35-60 ml) (Figure 3). The fractions relevant to the peaks were combined and freeze-dried. The sugar profile of fractions with relatively good yield is shown in Table 3.

According to Table 4, partial hydrolysis was able to break the pectin and HCII into oligosaccharides. Oligosaccharide from pectin contains 52.5% mannose, 32.2% galactose and 15.3% rhamnose. The partial hydrolysis of HCII was able to break the polysaccharide into an oligosaccharide having mannose as the major neutral sugar (95.6%) in the fraction. In addition to that, it also contained 2.7% glucose, 1.1% galactose and 0.6% xylose.

**Discussion**

The Cell wall polysaccharides of coconut kernel (CCWP) were fractionated into several polysaccharide fractions. The fractions are pectin which was extracted with 0.5% ammonium oxalate – oxalic acid, hemicellulose I (HCI) which was extracted with 4% NaOH, hemicellulose II (HCII), which was extracted with 10% NaOH, hemicellulose III (HCIII) which were concentrated with 17.5% NaOH and the remaining portion was the fraction consisting of cellulose which was isolated by washing with water and acetone. Several studies were carried out using similar methods to study polysaccharide structures of plant sources (Government et al., 2019; Xu et al., 2018; Ghosh et al., 2004).

According to Van soest (1963) hemicellulose is the difference between plant-based fiber that is insoluble in neutral detergent fiber solution (NDF) and insoluble in acid detergent solutions (ADF). According to the method of Van soest (1963) CCWP contained 41.6% (difference between NDF and ADF contents) hemicellulose (Yalegama et al., 2022). The present study of sequential extraction with NaOH collects hemicellulose as NaOH-soluble polysaccharides and a total of 41.6% of hemicellulose contained in CCWP (Table 1). The results indicated that the amount of hemicellulose obtained from neutral detergent and acid detergent methods and the recovery as NaOH soluble are the same.

According to Del Rosario and Gabuya (1980), 70% alcohol-insoluble matter of Makapuno coconut kernel

(variety of coconut having soft coconut kernel) contained 5.9% pectin, 8.7% HCl, 39.5% HCII, 2% HCIII and cellulose 22.2%. Makapuno coconut contains very low pectin and HCl concentrations compared to their concentrations of CCWP. HCII concentration of Makapuno coconut kernel is higher than the CCWP, while a very low concentration of HCIII is reported in both Makapuno and CCWP. CCWP was collected from Tall variety of coconut and has a harder kernel structure than Makapuno kernel. The difference in concentration of the fractionations of polysaccharides from CCWP and alcohol insoluble matter of Makapuno coconut kernel is the structural difference due to the variety of coconut.

The neutral sugar profile in Table 2 shows the absence of xylose and glucose in both pectin and HCII. Sugars that are not detected can remain with a fraction that is resistant to hydrolysis. Hemicellulose structures obtained by several methods showed different neutral monosaccharides under similar hydrolysis conditions. Neutral monosaccharides in the hemicellulose of CCWP as a result of acid detergent fiber soluble procedure contain rhamnose and glucose as major neutral monosaccharides (Yalegama et al., 2022). In contrast, HCl contains mannose as a major neutral sugar. HCII which is obtained under high alkali concentration contains rhamnose as the major sugar. Therefore, hemicellulose obtained from different methods contain different neutral sugar compositions, and therefore their structure could be different.

Hemicellulose of CCWP extracted from different concentrations of NaOH contains different sugar profiles (Table 2). According to Fu et al. (2018), alkaline solutions such as NaOH saponify naturally occurring insolubilizing ester linkages of uronic groups that cross-link the hemicellulose molecule with each other or with cellulose or lignin. Therefore, different concentrations of NaOH can release different types of molecules. Whistler (1993) stated that harsh treatments can break hemicellulose and decompose the released sugar. Therefore, increasing concentration may decompose some of the polysaccharides in the coconut kernel.

Several authors worked on hemicellulose, pectin and cellulose isolated from plant materials using alkaline conditions. According to Sun et al. (1999), a 5% NaOH soluble fraction of sago pith hemicellulose contains 9.36% rhamnose, 0.98% fucose, 16.3% arabinose, 32.45% xylose, 4.2% mannose, 24.43% glucose and 12.28% galactose. Twenty-four percent NaOH soluble fraction contained 4.07% rhamnose, 2.8% fucose, 3.18% arabinose, 3.07% mannose, 38.58% glucose and 15.0% galactose. The cellulose also contains several neutral monosaccharides; 2.97% rhamnose, 0.47% fucose, 1.78% arabinose, 3.45% xylose, 1.91% mannose, 88.99% glucose and 0.43% galactose. According to Eda et al. (1983) 5% KOH soluble fraction of tobacco cell cultures contains 2.9% rhamnose, 31.6% arabinose, 27.9% xylose, 6.4% mannose, 8.9% galactose, and 24.8% glucose. The KOH 24% contains 3% rhamnose, 14.8% arabinose, 16.2% xylose, 9.4% mannose, 24.6% galactose, 32% glucose. The cellulose fraction contains glucose (82.8%), 1.0% rhamnose, 4.5% arabinose, 3.3% xylose, 1.4% mannose and 7.0% galactose. The cellulose in the present study also contains several neutral

sugars in addition to glucose. Fu et al. (2018) mentioned that alkali extraction of polysaccharides causes the degradation of hemicellulose and some parts of oligosaccharides and monosaccharides. Therefore, some of the polysaccharides may remain in the cellulose fraction. The insoluble fractions in the solubilization process with different NaOH concentrations can be present in cellulose fraction. The solubilization of hemicellulose was carried out with 5, 10, and 17.5% NaOH. KOH of 24% was used to separate hemicellulose from tobacco cell culture (Eda et al., 1983) and reported a higher percentage of glucose.

According to the results, HCl and HCII of CCWP contained almost all the neutral sugars, which limits further study of the polysaccharide structures because of their compact nature. Therefore, partial hydrolysis of hemicellulose was done in order to obtain oligosaccharides. The partial hydrolysate was further concentrated using size exclusion chromatography (SEC) for the concentration of oligosaccharides.

Pectin-rich fraction of okra obtained as hot buffer soluble matter showed that it contained rhamnose (26%), galactose (34%), and glucose (1%) (Sengkhampan et al., 2009). The pectic substances obtained from unripe mango contained rhamnose, arabinose, xylose, mannose, galactose, and glucose. The same neutral sugars were present in the ripe mango. Due to the presence of rhamnose in the neutral sugar composition, they reported that pectin-derived molecule arabinogalactan side chains are linked to polygalacturonic acid main chains through rhamnose residue (Yashoda et al., 2005).

Partially hydrolyzed pectin of CCWP produced an oligosaccharide having 52% mannose, 32.2% galactose and 15.3% rhamnose. Hilz et al. (2006) reported that rhamnogalacturonan is present in all pectin substances. The sugar composition of pectin from CCWP also contains rhamnose and galactose. The fragment did not contain a considerable amount of arabinose, although it was originally present in the parent pectin (Table 1). The complete hydrolysis of pectin showed that it did not contain xylose and glucose at a detectable level and partial hydrolysate also showed the absence of xylose and glucose at a detectable level, confirming that pectin molecule does not contain xylose and glucose.

The partial hydrolysis of HCII was able to break the polysaccharide into an oligosaccharide having mannose as the major neutral sugar (Table 4). The sugar profile of partially hydrolyzed HCII indicates the presence of an oligosaccharide consisting of mannan as the major chain. According to the sugar profile, the oligosaccharide may be a galactoglucoman with xylose side chain. Galactoglucoman was isolated by Lundqvist et al. (2002) from spruce (*Picea abies*). Water soluble hemicellulose was separated from spruce by size exclusion chromatography on Sephadex G15 with galactose:glucose:mannose with 0.1:1:4. HCII isolated from coconut kernel contains major mannose side chain with Xylose:galactose:glucose:mannose with 1:1.8:4.5:159.3. The galactoglucoman is an oligosaccharide with prebiotic properties (Polari et al., 2012). As HCII partial hydrolysate contains xylose in addition to galactose, glucose and mannose, the oligosaccharide can be identified as xylo-oligosaccharide.

Xylo-oligosaccharide was separated from date palm using an alkaline solution, which was found to have prebiotic properties and, therefore can be used in functional food application. Ho et al. (2017) stated that xylo-oligosaccharides and xylo-polysaccharides with different molecular weights were reported to have shown the invitro fermentability of human fecal bacteria. The oligosaccharides isolated from CCWP have prebiotic properties and therefore, defatted desiccated coconut remaining from the virgin coconut oil process can be used to produce oligosaccharides having prebiotic properties.

## Conclusion

Dehydrated defatted coconut kernel, the by-product of coconut oil production, is a source of food-grade fiber. The cell wall polysaccharides obtained from dehydrated defatted coconut kernel contain pectin and hemicellulose, which can be further concentrated to extract oligosaccharides. An oligosaccharide having 52.5% mannose, 32.2% galactose and 15.3% rhamnose was concentrated from pectin while an oligosaccharide having mannose 95.6%, 2.7% glucose, 1.1% galactose and 0.6% xylose was concentrated with hemicelluloses extracted from 10% NaOH. The process developed in this study can be utilized to produce oligosaccharides from dehydrated defatted coconut kernel.

## Conflict of interest

The authors declare that there is no conflict of interest in publishing this article

## References

- Ataei, D., hamidi-Esfahani, Z., & Ahmadi-Gavlighi, H. (2020). Enzymatic production of xylooligosaccharide from date (*Phoenix dactylifera* L.) seed. *Food Science & Nutrition*, 8(12), 6699-6707.
- Coconut statistics (2021). *Annual publication of coconut Development Authority, Sri Lanka*.
- Coconut oil: global production volume 2012/13-2022/23. (2022, October 12). Retrieved from <http://www.statista.com/statistics/613147/coconut-oil-production-volume-worldwide>
- Del Rosario, R. R., & Gabuya, E. S. (1980). Preliminary studies on the polysaccharide composition of coconut and makapuno [mutant coconut] cell wall [study conducted at UPLB, Philippines]. *Philippine journal of coconut studies*, 5(1), 17-21.
- DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, 28(3), 350-356.
- Eda, S., Akiyama, Y., Katō, K., Ishizu, A., & Nakano, J. (1983). Methylation analysis of cell wall polysaccharides from suspension-cultured cells of *Nicotiana tabacum*. *Agricultural and biological chemistry*, 47(8), 1783-1789.
- Fu, G., Yue, P., Hu, Y., Li, N., Shi, Z., & Peng, F. (2018). Fractionation of DMSO-extracted and NaOH-extracted hemicelluloses by gradient ethanol precipitation from *Neosinocalamus affinis*. *International Journal of Polymer Science*, 2018.
- Ghosh, P., Ghosal, P., Thakur, S., Lerouge, P., Loutelier-Bourhis, C., Driouich, A., & Ray, B. (2004). Cell wall polysaccharides of *Brassica campestris* seed cake: isolation and structural features. *Carbohydrate polymers*, 57(1), 7-13.
- Olowokere, J. A., Odineze, C. M., Anidobu, C. O., Yerima, E. A., & Nnaemeka, B. I. (2019). Influence of soaking time and sodium hydroxide concentration on the chemical composition of treated mango seed shell flour for composite application. *Journal of Applied Sciences and Environmental Management*, 23(1), 21-28.
- Hilz, H., Williams, P., Doco, T., Schols, H. A., & Voragen, A. G. (2006). The pectic polysaccharide rhamnogalacturonan II is present as a dimer in pectic populations of bilberries and black currants in muro and in juice. *Carbohydrate polymers*, 65(4), 521-528.
- Ho, A. L., Kosik, O., Lovegrove, A., Charalampopoulos, D., & Rastall, R. A. (2018). In vitro fermentability of xylo-oligosaccharide and xylo-polysaccharide fractions with different molecular weights by human faecal bacteria. *Carbohydrate polymers*, 179, 50-58.
- Huang, L. Z., Ma, M. G., Ji, X. X., Choi, S. E., & Si, C. (2021). Recent developments and applications of hemicellulose from wheat straw: A review. *Frontiers in Bioengineering and Biotechnology*, 9, 690773.
- Ji, L., Jie, Z., Ying, X., Yue, Q., Zhou, Y., & Sun, L. (2018). Structural characterization of alkali-soluble polysaccharides from *Panax ginseng* CA Meyer. *Royal Society Open Science*, 5(3), 171644.
- Knez, Ž., Hrnčič, M. K., Čolnik, M., & Škerget, M. (2018). Chemicals and value added compounds from biomass using sub-and supercritical water. *The Journal of Supercritical Fluids*, 133, 591-602.
- Lundqvist, J., Teleman, A., Junel, L., Zacchi, G., Dahlman, O., Tjerneld, F., & Stålbrand, H. (2002). Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). *Carbohydrate Polymers*, 48(1), 29-39.
- Olmos, J. C., & Hansen, M. Z. (2012). Enzymatic depolymerization of sugar beet pulp: Production and characterization of pectin and pectic-oligosaccharides as a potential source for functional carbohydrates. *Chemical Engineering Journal*, 192, 29-36.
- Mohd Nor, N. A. N., Abbasiliasi, S., Marikkar, M. N., Ariff, A., Amid, M., Lamasudin, D. U., ... & Mustafa, S. (2017). Defatted coconut residue crude polysaccharides as potential prebiotics: study of their effects on proliferation and acidifying activity of probiotics in vitro. *Journal of food science and technology*, 54, 164-173.
- Price, N. P., Hartman, T. M., Faber, T. A., Vermillion, K. E., & Fahey Jr, G. C. (2011). Galactoglucomannan oligosaccharides (GGMO) from a molasses byproduct of pine (*Pinus taeda*) fiberboard production. *Journal of agricultural and food chemistry*, 59(5), 1854-1861.
- Polari, L., Ojansivu, P., Mäkelä, S., Eckerman, C., Holmbom, B., & Salminen, S. (2012). Galactoglucomannan extracted from spruce (*Picea abies*) as a carbohydrate source for probiotic bacteria. *Journal of agricultural and food chemistry*, 60(44), 11037-11043.
- Sengkhamparn, N., Verhoef, R., Schols, H. A., Sajjanantakul, T., & Voragen, A. G. (2009). Characterisation of cell wall polysaccharides from okra (*Abelmoschus esculentus* (L.) Moench). *Carbohydrate Research*, 344(14), 1824-1832.

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- Sritrakul, N., & Keawsompong, S. (2021). Polysaccharides in copra meal: extraction conditions, optimisation and characterisation. *Int J Agric Technol*, 17, 337-348.
- Sun, R., Jones, G. L., Tomkinson, J., & Bolton, J. (1999). Fractional isolation and partial characterization of non-starch polysaccharides and lignin from sago pith. *Industrial Crops and Products*, 9(3), 211-220.
- Van Audenhove, J., Bernaerts, T., De Smet, V., Delbaere, S., Van Loey, A. M., & Hendrickx, M. E. (2021). The structure and composition of extracted pectin and residual cell wall material from processing tomato: The role of a stepwise approach versus high-pressure homogenization-facilitated acid extraction. *Foods*, 10(5), 1064.
- Soest, P. V. (1963). Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *Journal of the Association of official Agricultural Chemists*, 46(5), 829-835.
- Xu, J. D., Li, M. F., & Sun, R. C. (2018). Successive fractionations of hemicelluloses and lignin from sorghum stem by sodium hydroxide aqueous solutions with increased concentrations. *BioResources*, 13(2), 2356-2373.
- Yashoda, H. M., Prabha, T. N., & Tharanathan, R. N. (2005). Mango ripening—chemical and structural characterization of pectic and hemicellulosic polysaccharides. *Carbohydrate Research*, 340(7), 1335-1342.
- WHISTLER, R. L. (1993). Hemicelluloses. In *Industrial gums* (pp. 295-308). Academic Press.
- Yalegama, L. L. W. C., Karunaratne, D. N., & Sivakanesan, R. (2022). A Study on Acid Hydrolysis and Composition of Polysaccharides Concentrated from Coconut Kernel. *CARD*, 38, 30-38.