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A Study on Acid Hydrolysis and Composition of Polysaccharides Concentrated from Coconut Kernel

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Abstract

Defatted dehydrated coconut kernel powder (DDCP) is the by-product obtained from virgin coconut oil production through dry process. The aim of the study was to concentrate polysaccharides from DDCP and to investigate their acid hydrolysis capacity and the monosaccharides composition. Residual fat, protein and soluble sugars of DDCP were removed to concentrate coconut kernel Insoluble polysaccharides (CKIP) while water extract of DDCP was used to concentrate coconut kernel soluble polysaccharides (CKSP). Neutral detergent solution (NDS) was used to concentrate neutral detergent soluble polysaccharides (NDSP) and neutral detergent insoluble polysaccharides (NDIP) from CKIP. The acid detergent solution (ADS) was used to concentrate acid detergent soluble polysaccharides (ADSP) and acid detergent insoluble polysaccharides (ADIP) from CKIP. Results indicated fresh coconut kernel contained 7.2±1.5% carbohydrates and the content increased to 78.1±1.3% with the removal of residual fat, protein and sugars. The yields of the polysaccharide fractions were 46.0±3.1% (CKIP) and 9.2± 0.1% (CKSP), 10.2±0.3% (NDSP) and 78.3±4.2% (NDIP), 25.1±0.3% (ADSP) and 45.2±2.9% (ADIP). Trifluoracetic acid had a higher hydrolyzing capacity than sulphuric acid except for hydrolyzing of ADIP. The monosaccharides composition of the polysaccharides was significantly different ($p<0.05$) among the polysaccharide concentrates. The main monosaccharides in NDSP were glucose (73.86%) and xylose (19.7%) and, in ADSP were rhamnose (33.45%) and glucose (46.91%). Rhamnose (29.95%) arabinose (26.38%), xylose (21.56%) and mannose (12.87%) were present in CKSP while mannose (68.46%), galactose (20.59%) and xylose (10.59%) were present in CKIP. Results indicated that soluble polysaccharides of coconut kernel were hydrolyzed into monosaccharides readily compared to the insoluble polysaccharides.

Keywords: Coconut kernel, soluble polysaccharide, insoluble polysaccharides, acid hydrolysis, monosaccharide composition

Introduction

Coconut kernel is an important commodity in food preparation and is a raw material for various food-related industries. Coconut oil and coconut milk are common coconut kernel-based products. Coconut oil production generates by-product of defatted coconut kernels which is mainly used for animal feed. However, these defatted coconut kernels are used for human consumption with improved technology for coconut oil production.

The coconut kernel contains 34-40% fat which is used for domestic cooking, bakery industry, soap and cosmetic industry, margarine-related industries, pharmaceutical industries and traditional medicines. Dehydrated coconut kernel contains 65- 70% fat which can be expelled by leaving nearly 40% of defatted coconut kernels. Therefore, a large amount of defatted coconut kernels is produced in coconut-producing countries like the Philippines, Indonesia, India and Sri Lanka. World coconut oil production is around 3.5 million metric tons (APCC, 2019). Therefore, approximately 2.1 million metric tons of defatted coconut kernels are available for further processing. The defatted coconut kernel produced in the coconut oil industry contains 53.10±0.54% carbohydrates, 4.30±0.01% fat, 25.6±0.2% protein, 5.79±4.74% mineral and 8.8% moisture when it is processed for animal feed (Pavitra *et al*., 2019). Coconut flour produced from defatted coconut kernel has been incorporated into rice or wheat flour to increase

the food value of bread, cookies and muffin (Beegum *et al*., 2016; Hewa Pathirana *et al*., 2020 & Ramaswamy & Sinthuja, 2013). However, limited studies have been conducted to utilize defatted coconut kernel as a source of polysaccharides or a food-grade fibre.

Food residues with high carbohydrate content are considered sources of energy and food ingredients (Gosavi *et al*., 2017). Vegetable and fruit wastes are potential sources for the production of food-grade fibres, pectin concentrates and bioethanol (Maurya *et al*., 2015;). Breaking down of polysaccharide structure of biomass is needed to produce value-added products like oligosaccharides, monosaccharides, disaccharides and nutraceuticals.

Coconut defatted kernel can be used to obtain polysaccharides to use as an alternative energy source. In addition, polysaccharides could be used to produce monosaccharides, disaccharides and oligosaccharides. Value addition to by-products of coconut oil is very essential to get more income for a sustainable coconut industry. Thongsook and Chaijamrus (2014) modified the physiochemical properties of copra meal, the defatted by-product from coconut oil expelling, using 0.5% HCl to increase its utilization in food applications as a low calory bulk ingredient. Modified copra meal showed improvement in qualities in bread and biscuits.

The objective of this study is to concentrate polysaccharide fractions from defatted coconut kernel which can be hydrolyzed into simple polysaccharides with low-cost techniques such as acid hydrolysis. Coconut kernel polysaccharides have shown prebiotic characteristics (Abbasiliasi *et al*., 2019, Mohd Nor *et al*., 2017). Therefore, coconut kernel polysaccharides can be incorporated into food to increase fibre content. Hydrolyzed polysaccharides have more applications in food, pharmaceutical and chemical industries as oligosaccharides, disaccharides and monosaccharides. Due to the compact nature of raw defatted coconut kernel, the acid cannot reach the polysaccharide to hydrolyze them (Becker *et al*., 2021). Therefore, the raw coconut defatted kernels must be treated with several reagents to remove residual components such as fat, protein and simple sugars to increase the value of fibre and can be processed for variety of polysaccharides isolates.

The findings of this study are benefitted by coconut industry by increasing the value of coconut in the production chain as well as the food and feed industry for the production of food supplemented with coconut-based food grade fibre.

Material and Methods

Materials

Coconuts: Fully mature coconuts were obtained from Bandirippuwa Estate of coconut Research Institute, Lunuwila, Sri Lanka. The coconut was the raw material for the preparation of dehydrated coconut kernel (DCK). Defatted DCK was used to obtain defatted desiccated coconut kernel powder (DDCP) which was the main raw material used in this study.

Reagents

1. Neutral detergent fibre solution (NDS) was prepared according to the methods reported by Vansoest (1963).
2. Acid detergent fibre solution (ADS) was prepared according to the method reported by Vansoest (1963).
3. All other chemicals were of analytical grade unless otherwise specified.

Methods

Dehydrated Coconut Kernel and dehydrated defatted coconut kernel powder: The husks of fully mature coconuts were removed. The outer shells were removed followed by peeling off brown skins. The white kernels were washed with potable water and were left for draining of excess water. The white coconut kernels (CK) were disintegrated to 0.5-1mm thickness and then dehydrated using a cabinet-type dehydrator (Wessburg, Martin; Germany) at 60-65°C. The dehydrated coconut kernels (DCK) were expelled (DD-85 Komet; cold press expeller, Germany) to remove oil and the defatted kernels (by-product) was ground using a laboratory grinder (LG, Korea) into a fine powder. The powder was further defatted using chloroform (1:5 w/v) to yield dehydrated defatted coconut kernel powder (DDCP).

Coconut kernel insoluble polysaccharides: To DDCP (100g), a solution of 0.1N NaOH (500ml) was added and the contents were stirred for two hours. Then the insoluble residue was collected by filtration through double layers of linen cloth. The residue was washed with distilled water until washings were neutral to phenolphthalein. A 500ml of 70% aqueous ethanol was added to the residue and heated to 80°C for 30 min while mixing. The insoluble material in hot aqueous ethanol was collected by filtration through a double layer of linen cloth. The residue remaining (CKIP) after the above procedure was washed with 70% ethanol. CKIP was dried at 70°C.

Coconut Kernel Soluble Polysaccharides: DDCP (100g) was mixed with 400ml of distilled water and allowed to mix for 3 hours. The water extract separated by using a hydraulic pressing juice extractor (Sakaya, Thailand) was added to 4 volumes of ethanol (Proskey *et al.*, 1995; Bao *et al.*, 2001) and left overnight at 4°C to precipitate the water-soluble fibre. After that, the precipitate was collected by centrifugation at 5,000 rpm. The precipitate was freeze-dried to obtain coconut kernel soluble polysaccharide (CKSP).

Concentration of Neutral Detergent Insoluble Polysaccharides of coconut kernel: Coconut kernel insoluble polysaccharides (CKIP) were ground to pass 1mm standard wire mesh (screen). The ground sample (5g) was placed in a round bottom flask with a fitted reflux condenser. Neutral detergent fibre solution (NDS) (500ml) was added to the flask with 0.5g of sodium sulphite and a few drops of n- octanol. The contents were heated and boiled for 60 minutes. After 60 minutes, the soluble and insoluble components were separated by filtration through a cheesecloth. The filtrate was collected separately. The insoluble material (retentate) was washed 3 times with hot distilled water and twice with cold acetone and dehydrated for 8 hours at 105°C to obtain neutral detergent insoluble polysaccharides (NDIP) of coconut kernel.

Concentration of Neutral Detergent Soluble Polysaccharides of coconut kernel: The filtrate of NDIP contains soluble components due to the action of NDS. The filtrate of NDIP was added to 4 volumes of ethanol (Proskey *et al.*, 1995; Bao *et al.*, 2001) and left overnight for precipitation of neutral detergent soluble polysaccharides. The precipitate was collected by centrifugation at 5,000 rpm. The precipitate was freeze-dried to obtain Neutral detergent soluble polysaccharides (NDSP) of coconut kernel.

Concentration of Acid Detergent Insoluble Polysaccharides of coconut kernel: CKIP were ground to pass a 1 mm standard sieve. The ground sample (5g) was weighed into a round bottom flask with a fitted reflux condenser. Then 500ml of Acid detergent fibre solution (ADS) and 10ml of deca-hydro naphthalene were added to the flask. The contents were heated to boiling and the boiling was continued for 60 minutes. After 60 minutes, the soluble and insoluble components were separated by filtration through a cheesecloth. The filtrate was collected separately. The insoluble matter (retentate) in the ADF solution was washed with hot water and then with acetone to isolate insoluble polysaccharides (ADIP) in ADS. ADIP of coconut kernel was dried at 70°C.

Concentration of Acid Detergent Soluble Polysaccharides of coconut kernel: The filtrate of ADIP was added to 4 volumes of ethanol (Proskey *et al.*, 1995; Bao *et al.*, 2001). The contents were left overnight for precipitation of acid detergent soluble polysaccharides. The precipitate was collected by centrifugation at 5,000 rpm, and freeze-dried to obtain Acid Detergent Soluble Polysaccharides (ADSP) of Coconut kernel.

Proximate composition: Proximate compositions of fresh coconut kernel, dehydrated coconut kernel, defatted dehydrated coconut kernel powder and coconut kernel insoluble polysaccharides were determined using the AOAC official method (AOAC, 1990). Carbohydrate composition was calculated by subtracting percentages of moisture, fat, protein, sugar and ash from 100.0.

Determination of the effect of different acids for hydrolysis of polysaccharides

Hydrolysis with Trifluoroacetic acid

The Sample was placed in a flat bottom flask (0.1g of CKSP, NDSP, ADSP and 0.05g of CKIP, NDIP and ADIP). 2M Trifluoroacetic acid (TFA) (10 ml) was added and heated under reflux for 1 hour. Then the contents were cooled to room temperature and filtered through a Whatman 42 filter paper. The filtrate was analyzed for total sugar by phenol sulphuric acid method (Dubois *et al.*, 1956). The experiment was repeated by digesting samples for 2,4,6,8,10 and 12 hours of heating under reflux. Each analysis was carried out in triplicate and the mean values of the total sugar were calculated. Hydrolysis at t=0 was considered as control.

Hydrolysis with Sulphuric acid

The Sample (0.1g of CKSP, NDSP, ADSP and 0.05g of CKIP NDIP and ADIP) was mixed with 1.25ml of 72% (w/w) sulphuric acid with a glass stick for 15 minutes at room temperature. The contents were then diluted with 13.5ml of water and refluxed for 1 hour. At the end of 1 hour, the contents were cooled to room temperature and 3.1ml of 32% NaOH solution was added. The contents were filtered through a Whatman 42 filter paper (Hoebler *et al.*, 1989). The sugar content was determined using the phenol sulphuric acid method (Dubois *et al.*, 1956). The experiment was repeated with 2, 4, 6, 8, 10 and 12 hours of heating under reflux. Each analysis was carried out in triplicates and the mean values were calculated.

The amount of sugar released due to hydrolysis without refluxing was considered as initial sugar hydrolyzed. Time vs the sugar concentration of hydrolysate was plotted and the hydrolyzing capacity of the two acids were compared.

Determination of monosaccharide composition of the hydrolysate

Derivatization of alditol acetates of neutral sugars in cell wall polysaccharides

The cell wall polysaccharides (CKIP, CKSP, NDSP and ADSP) were hydrolyzed separately in 2M TFA (0.1 g of sample in 10 ml of acid) at 100 oC for 14-18 hours. The hydrolysate was filtered through 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

Alditol acetates were analyzed using Gas Chromatograph (Agilent 4890D, Agilent Technologies (Pvt) Ltd., USA), Column–DB 23.3mm x 0.32mm x 0.25µm film thickness, Injection volume–1.0µl, 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 1µl 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.

Experimental design: All experiments were conducted in a completely randomized design. Mean values and standard deviations were calculated. A comparison of means was carried out with T-test and values with $p<0.05$ were considered significant.

Results

Proximate composition of coconut kernel at various processing stages

Coconut kernel is a rich source of fat. After the oil is extracted, other components; sugar, mineral, protein and

Table 1. Summary statistics for variables in the production function and Inefficiency model

Constituent	Composition %			
	Fresh coconut kernel	Dehydrated coconut kernel	Dehydrated defatted coconut kernel	Coconut kernel insoluble polysaccharide
Moisture (water)	41.7±0.2	2.5±0.1	4.2±0.2	6.7±0.1
Fat	40.2±0.4	65.5±0.3	9.2±0.3	2.8±0.1
Protein	4.1±0.2	6.8±0.2	12.6±0.3	11.9±0.9
Sugar	5.6±0.2	6.5±0.2	13.7±0.3	0.4±0.1
Ash	1.2±0.1	3.5±0.1	8.2±0.5	0.1±0.01
Carbohydrates by difference	7.2±1.5	15.2±1.5	52.1±2.2	78.1±1.3

Values are mean ± SD of triplicate analysis

carbohydrates are concentrated in the defatted coconut kernel. The proximate compositions of fresh coconut kernel (FCK), dehydrated coconut kernel (DCK), defatted dehydrated coconut kernel powder (DDCP) and coconut kernel insoluble polysaccharides (CKIP) are given in Table 1.

The fat which is 40.2±0.4% in fresh kernel gets concentrated to 65.5±0.3% after dehydration. When the dehydrated kernel is mechanically expelled to separate oil the fat content is reduced to 9.2±0.3%, while carbohydrates increase from 7.2±1.5% in the fresh kernel to 52.1±2.3% in the defatted dehydrated coconut kernel. Further, with the removal of protein and soluble sugars, carbohydrate increases to 78.1±1.3% in CKIP. In this paper, attention has been given to the concentration of carbohydrates, which are the cell wall polysaccharides of the coconut kernel.

The protein content increases from 4.1±0.2 in fresh coconut kernel to 12.6±0.3 in defatted coconut kernel after oil is expelled. However, the removal of protein using 0.1 M NaOH has not reduced protein content significantly.

Fractionation of coconut kernel insoluble polysaccharides

Figure 1 illustrates the concentration of soluble and insoluble polysaccharides of DDCP. The percentage yields of the different types of polysaccharide fractions of defatted dehydrated coconut kernel (DDCP) are given in Table 2.

The DDCP is further concentrated by removing fat, protein and soluble sugars to obtain 46.0±3.1% Coconut Kernel Insoluble Polysaccharide (CKIP).

Coconut kernel soluble polysaccharide (CKSP) accounts for the soluble carbohydrates, proteins and minerals in the water extract of DDCP, which is 9.2 ±0.1%. The materials which are precipitated in alcohol were isolated as CKSP (Table 2).

The CKIP isolate is fractionated into NDIP (78.3±4.2%) and NDSP (10.2±0.3%) (Table 2). CKIP isolate does not have

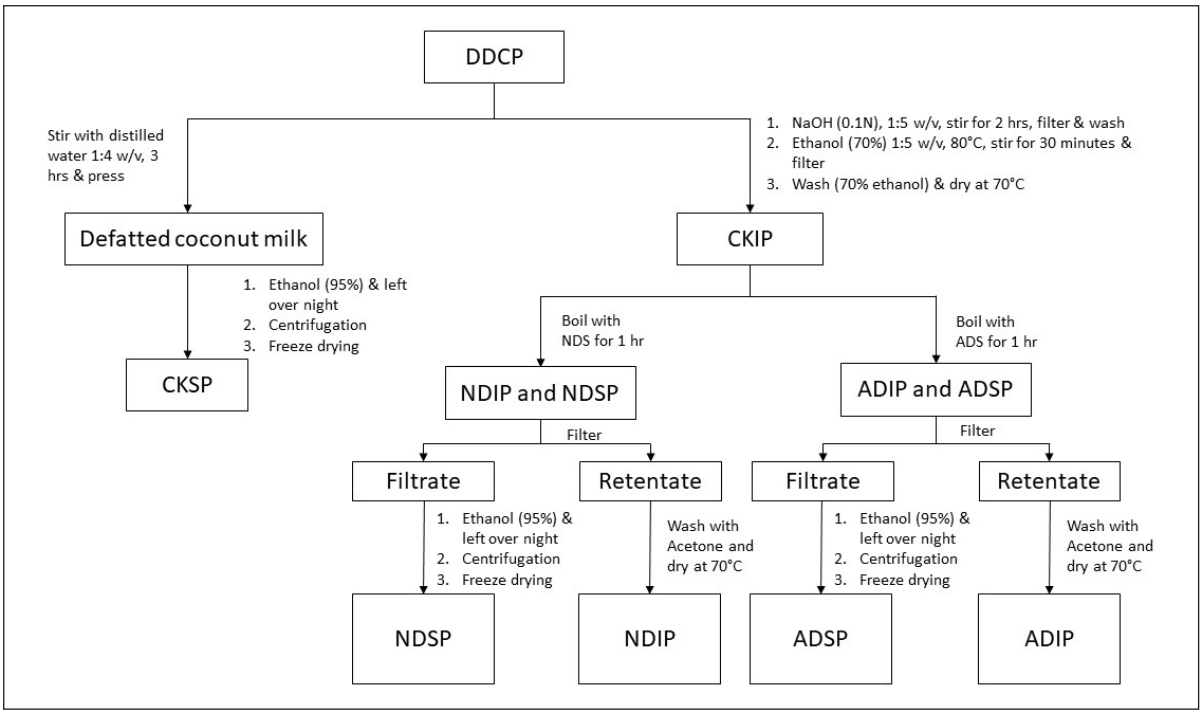

Figure 1. Concentration of coconut kernel polysaccharides

considerable content of soluble sugars (Table 1). Most of the proteins, oligosaccharides and minerals are washed during the isolation of NDIP. CKIP contains lower ADIP content than the content of NDIP. The amount of ADSP was higher than the NDSP. According to Table 2, the total of ADIP and ADSP is 70.3 ± 4% of the CKIP of coconut kernel.

Coconut kernel soluble polysaccharide (CKSP) accounts for the soluble carbohydrates, proteins and minerals in the water extract of DDCP, which is 9.2 ±0.1%. The materials which are precipitated in alcohol are isolated as CKSP (Table 2).

The CKIP isolate is fractionated into NDIP (78.3±4.2%) and NDSP (10.2±0.3%) (Table 2). CKIP isolate does not have considerable content of soluble sugars (Table 1). Most of the proteins, oligosaccharides and minerals are washed during the isolation of NDIP. CKIP contains lower ADIP content than the content of NDIP. The amount of ADSP was higher than the NDSP. According to Table 2, the total of ADIP and ADSP was 70.3 ± 4% of the CKIP of coconut kernel.

Hydrolysis of polysaccharides CKIP and CKSP

Sugar concentrations formed due to hydrolysis of CKIP and CKSP with TFA and sulphuric acid with time are shown in Figure 2. The zero in the time scale shows the amount of sugar released due to the addition of reagent before heat treatment. The concentration of sugars in the hydrolysates of CKIP with sulphuric acid did not increase significantly (P>0.05) during 10 hours of hydrolysis. In contrast, the concentration of sugars increased significantly (p<0.05) due to hydrolysis of CKIP by TFA after one hour compared to the initial point followed by the production of similar amounts at each time interval after one hour.

TFA produced significantly higher concentrations (p<0.05) of sugars due to hydrolysis of CKIP (25.7-32.08 ppm) compared to the amount of sugar produced by sulphuric acid (13.03-20.7 ppm).

The concentration was not significant (p>0.05) until 2 hours when sulphuric acid was used to hydrolyze CKSP. The total sugars produced by sulphuric acid were 60-125 ppm while that by TFA was 75-200 ppm. It was observed that soluble polysaccharides dissolved readily in sulphuric acid and TFA compared to the insoluble polysaccharides. The amount of sugar increased with time when both acids were used, indicating the hydrolysis had to be continued for more than 12 hours for the complete hydrolysis. TFA produced significantly higher sugar concentrations (p<0.05) due to hydrolysis than sulphuric acid did.

Table 2. Percentage yield of different polysaccharides concentrated from defatted desiccated coconut kernel powder

Material	Polysaccharides	Yield
DDCP	Coconut kernel insoluble polysaccharides (CKIP)	46.0±3.1%
	Coconut kernel soluble polysaccharides (CKSP)	09.2 ±0.1%
	Total insoluble and soluble polysaccharides in coconut kernel	55.2±3.2%
CKIP	Neutral detergent Soluble polysaccharides (NDSP)	10.2±0.3%
	Neutral detergent Insoluble polysaccharides (NDIP)	78.3±4.2%
	Total soluble and insoluble polysaccharides of coconut kernel due to NDS	88.5±4.5%
CKIP	Acid detergent Soluble polysaccharides (ADSP)	25.1±1.6%
	Acid detergent insoluble polysaccharides (ADIP)	45.2± 2.9%
	Total soluble and insoluble polysaccharides of coconut kernel due to ADS	70.3±4.5%

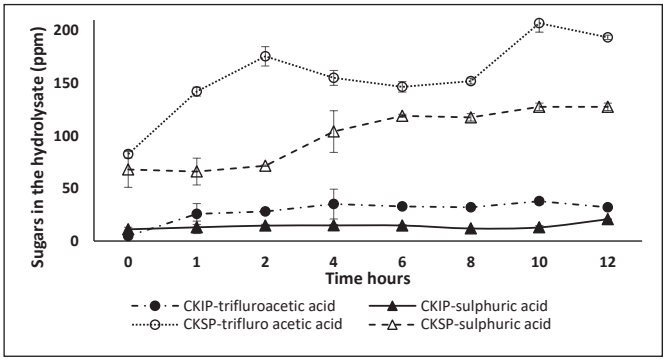

Figure 2. Hydrolysis of coconut kernel insoluble polysaccharides and coconut kernel soluble polysaccharides with time

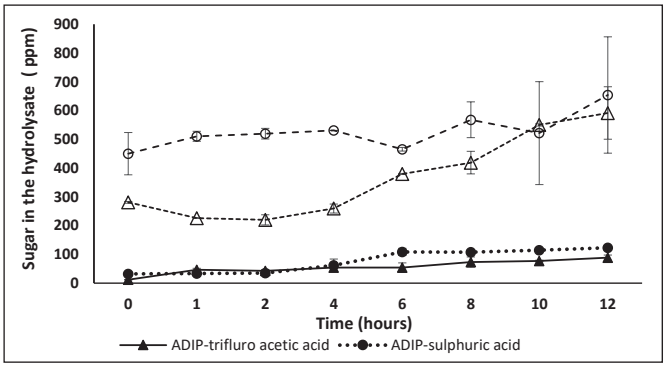

Figure 3. Hydrolysis of acid detergent insoluble polysaccharides and acid detergent soluble polysaccharides with time

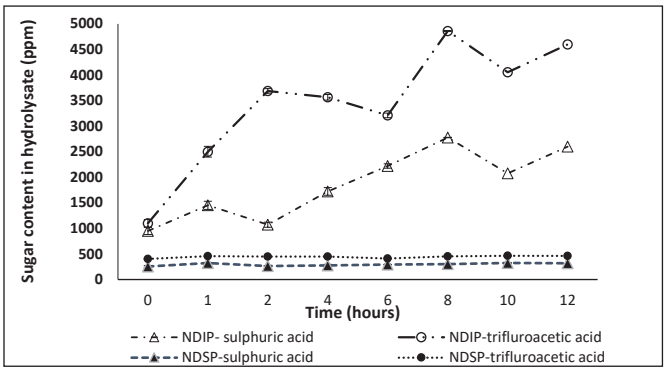

Figure 4. Hydrolysis of neutral detergent insoluble polysaccharides and neutral detergent soluble polysaccharides with time

Hydrolysis of ADIP and ADSP coconut kernel

Figure 3 shows the concentration of sugars produced with time due to hydrolysis of ADIP and ADSP of coconut kernel in the presence of TFA and sulphuric acid.

Figure 3 shows a significant increase in sugar concentrations with time when ADIP and ADSP of coconut kernel were hydrolyzed with both TFA and sulphuric acid. The trends are parallel to the hydrolysis of CKIP and CKSP. The sugar concentration of the hydrolysate of ADIP due to hydrolysis by both TFA and sulphuric acid did not significantly change between 1-4 hours ($p>0.05$) and then, a significant increase in sugar concentration was observed after 6 hours. Similarly, Sulphuric acid produced a parallel trend of hydrolysis but by releasing higher concentrations after 6 hours (108.6–123.0 ppm) compared to 53.8–88.6 ppm released by TFA.

According to figure 3, ADSP of coconut kernel is readily hydrolyzed compared to ADIP. Therefore, ADSP has bonds that are easily hydrolyzed compared to insoluble polysaccharides. Hydrolysis of ADSP with TFA produced significantly higher sugar concentration ($p<0.05$) ranging between 450.07 ppm and 568.0 ppm compared to sulphuric acid (280.8 - 419 ppm) until 8 hours. However, when hydrolysis was continued for more than 8 hours, both acids released more or less similar amounts of sugars indicating both acids had similar hydrolyzing capacity for ADSP with longer durations.

Hydrolysis of NDIP and NDSP of coconut kernel

The figure 4 shows the amounts of sugars formed due to hydrolysis of NDIP and NDSP of coconut kernel using sulphuric acid and TFA with time.

The data showed that significantly higher sugar concentrations ($p<0.05$) were released by hydrolysis of NDIP and NDSP by both TFA and sulphuric acid compared to sugar released by hydrolysis of CKIP, CKSP, ADIP and ADSP by both TFA and sulphuric acid (Figure 2, 3 & 4). In contrast to other isolates, NDIP had higher hydrolyzing power than its soluble polysaccharide counterpart.

TFA produced a significantly higher concentration ($p<0.05$) of sugars (1100–4600 ppm) due to hydrolysis of NDIP compared to sulphuric acid (950–2600 ppm) during 12 hours period. NDSP hydrolyzed significantly lower sugar amounts (255–460 ppm) than NDIP (1100–4600 ppm).

Neutral sugar composition of polysaccharide isolates

The monosaccharide composition of the hydrolysate of the NDSP, ADSP CKIP and CKSP are given in table 3.

Hydrolysates of CKIP, CKSP, NDSP and ADSP contain significantly differing ($p<0.05$) monosaccharides composition of rhamnose, arabinose, xylose, mannose, galactose and glucose (Table 3). The hydrolysate of CKIP contains only xylose (10.59%), mannose (68.46%) and galactose (20.59%). CKSP produced more monosaccharides in the hydrolysate of TFA. It contained rhamnose (29.95%), arabinose (26.38%), xylose (21.56%) mannose (12.87%), glucose (9.2%) and trace of galactose. NDSP produced glucose (73.86%) followed by xylose of 19.7%, arabinose 2.69% and very low concentrations mannose, galactose and rhamnose (1.83%, 1.33% and 0.59% respectively) whereas ADSP is composed of Rhamnose (33.45%), glucose (46.91%), mannose (9.43%), arabinose (3.6%) and galactose (2.36%) and trace amounts of xylose.

Discussion

Polysaccharides of the coconut kernel are concentrated due to oil extraction to produce DDCP without any chemical treatment. Further concentration is possible by removing residual fat, protein and sugar to produce CKIP. The concentration of polysaccharides has been carried out using several organic and aqueous solutions. Among them are hot buffers, chelating agents, dilute and concentrated alkali (Shi, 2016; Im & Yoon, 2015). Insoluble polysaccharides are easily concentrated by dissolving fat, protein sugar and soluble matter from the raw polysaccharides. The concentration of soluble polysaccharides, soluble dietary fibre and pectin should be extracted into aqueous or organic solutions followed by precipitation using 85–96% ethanol (Guo *et al*., 2019; Bao

Table 3. Monosaccharides in the hydrolysate of neural detergent soluble polysaccharides, acid detergent soluble polysaccharides, coconut kernel insoluble polysaccharides and coconut kernel soluble polysaccharides

Neutral sugar	NDSP	ADSP	CKIP	CKSP
Rhamnose%	0.59c	33.45a	ND	29.95b
Arabinose%	2.69 c	3.6 b	ND	26.38a
Xylose%	19.7b	0.19d	10.59c	21.56a
Mannose%	1.83d	9.43c	68.46a	12.87b
Galactose%	1.33c	2.36b	20.59a	trace
Glucose%	73.86a	46.91b	ND	9.2c

NDSP– neutral detergent soluble polysaccharides;
ADSP– acid detergent soluble polysaccharides;
CKIP coconut kernel insoluble polysaccharides;
CKSP– coconut kernel soluble polysaccharides. ND– not detected;
Different superscripts in rows significantly different at $p<0.05$

et al., 2001; Mohd Nor *et al*., 2017). In this study, several solutions have been used to solubilize residual matter in CKIP while retaining soluble and insoluble polysaccharides.

CKIP was isolated using chloroform, 70% alcohol and 0.1M NaOH to remove residual fat, sugar and protein in this study. The selection of chloroform to remove residual fat justifies as it removes almost 70% of the fat present in DDCP (Table1). However, other workers used 80% alcohol to de-fat plant material in isolating carbohydrates (Shi, 2016). NaOH is the effective solution to remove residual protein (Yalegama and Chavan 2006) Neutral detergent fibre solution (NDS) was used to solubilize short chain polysaccharides and residual protein bonded to cell wall polysaccharides of CKIP (Vansoest,1963; Chen & Anderson, 1981). The insoluble component of CKIP in NDS was referred to as Neutral Detergent Insoluble Polysaccharides (NDIP). The soluble matter in NDS was isolated using 96% ethanol to isolate soluble polysaccharides and referred to as Neutral Detergent Soluble Polysaccharides (NDSP). Similarly, the insoluble and soluble polysaccharides of the CKIP were concentrated using Acid Detergent solvent (ADS) to isolate Acid Detergent Insoluble Polysaccharides (ADIP) and Acid Detergent Soluble Polysaccharides (ADSP), respectively. Acid detergent solution which can concentrate lignin, cellulose and acid-insoluble hemicelluloses, was developed for the estimation of acid resistant non-digestible material from the plant sources (Chen & Anderson, 1981). The hemicelluloses removed with the ADS contained acid-soluble hemicelluloses that were retained in ADSP (Gosavi *et al*., 2017; Fahey *et al*., 2019). The methods for isolation of NDIP and ADIP are based on the analytical procedure to determine neutral detergent fibre and acid detergent fibre from previous studies. However, studies are limited on the use of NDS and ADS to concentrate polysaccharides for food purposes. The present study shows that there is a potential for concentrating soluble and insoluble polysaccharides in coconut kernel with NDS and ADS.

The hydrolysis capacity of insoluble polysaccharides, CKIP and ADIP are very weak compared to the respective soluble polysaccharides. In contrast, NDIP is easily hydrolyzed to simple monosaccharides easily. Chen and Anderson (1981) reported that TFA is more efficient for hydrolysis of insoluble fibre of vegetable sources than sulphuric acid. This result was comparable to hydrolysis of both NDIP and CKIP of coconut kernel because TFA produced more sugar hydrolyzing NDIP and CKIP by TFA.

TFA releases more hexoses and pentoses than sulphuric acid from the insoluble cell wall polysaccharides (Sun *et al*., 1999) corroborates our study as more sugars were obtained from the TFA hydrolysis of insoluble polysaccharides than by sulphuric acid. Soluble cell wall polysaccharides extracted with hot water were hydrolyzed more readily by sulphuric acid than TFA (Chen and Anderson, 1981). In contrast, coconut kernel soluble fibre was more easily hydrolyzed by TFA than sulphuric acid due to the difference in the method of concentrating. Studies indicated that polysaccharides obtained from neutral detergent fibre solution contain acid-soluble hemicelluloses (Vansoest, 1963). Similarly, NDIP of coconut kernel, in the present study, contains acid-soluble hemicelluloses. The difference in formation sugars may be due to the easily hydrolysable sugars in NDIP of coconut kernel.

A study showed that hydrolysis of polysaccharides by 72% sulphuric acid followed by 8% sulphuric for 10 hours is equivalent to 2M TFA at 120oC for 1 hour. TFA is more suitable for hydrolysis of soluble polysaccharides than insoluble polysaccharides. Fractions of polysaccharides, not hydrolyzed, are the resistant portion for the reagent used for hydrolysis. According to Becker *et al*. (2021), sulphuric acid can reach the crystalline region of the pure polysaccharides to hydrolyze them. However, strong acid conditions may need to completely hydrolyze the polysaccharide structure. In a heterogenized system like in the present study, readily accessible domains of the polysaccharides will react fast to release monosaccharides into the acid medium. Some of the polysaccharides may be partially hydrolyzed into simpler polysaccharides. The hydrolyzing capacity may also depend on particle size, reaction time and temperature, and the polysaccharide source (Hoebler *et al*., 1989). Therefore, depending on the strength of acid and particle size of the polysaccharide, time and temperature, some of the polysaccharides may not be hydrolyzed.

The molar ratio of rhamnose: arabinose: xylose: mannose: galactose: glucose of NDSP is 1.0: 4.5: 33.0: 3.0: 2.2: 123.1. According to the molar ratios, NDSP is predominant with soluble glucan or xylose-glucan. The molar ratio of rhamnose: arabinose: xylose: mannose: galactose: glucose in the hydrolysate of ADSP is 167: 18: 1: 47: 12: 234.5 indicating the presence of soluble glucan or rhamnoglucan polysaccharide in ADSP. A higher ratio of mannose compared to galactose indicates the presence of galactomannan-based polysaccharides in ADSP.

The major neutral sugar present in the hydrolysate of CKIP was mannose consisting of 68.46% (Table 3) followed by galactose at 20.56% and xylose10.56% with xylose: mannose: galactose of 1:6:2. However, CKIP was not hydrolyzed incompletely (Figure 2) due to compact nature (Becker *et al*., 2021) and therefore, neutral sugars rhamnose, arabinose and glucose may have remained in the residual components. Further, hydrolysis of CKIP reached a constant after about 6 hours (the amount released was not significantly different after 6 hours) and therefore, the conditions were not sufficient to release a fragment with rhamnose, arabinose and glucose. The CKIP contains 11.9% of protein as a residual matter which contributes to the release of less neutral sugars and therefore polysaccharide is not pure enough for detailed studies. The cell wall polysaccharides present in CKIP show resistance to hydrolysis in the presence of sulphuric and trifluoracetic acid as they produced less than 40 ppm of sugar during hydrolysis.

The neutral sugar composition of water-soluble polysaccharides of maturing and mature coconut kernel has been studied using an enzymatic method to break β-D-glucosidic, α-galactosidic and β-mannosidic linkages. The study concluded the presence of 25% galactose and 75% mannose which indicated the presence of galactomannan (Balasubramanium, 1976). According to Saittagaroon *et al*. (1983), polysaccharides extracted from coconut

poonac (defatted copra) using hot water composed of 60% mannose, 17% galactose, 23% glucose and trace amounts of arabinose and xylose. Highly concentrated water-soluble polysaccharides of coconut kernel contained galactose, glucose and xylose as major monosaccharides (Mohr Nor *et al*., 2017). The observation partially agreed with the neutral sugar composition of CKSP and the difference is due to the difference in concentration of soluble polysaccharides

The polysaccharide fractions concentrated from defatted desiccated coconut powder consist of several short-chain oligosaccharides. The short chain oligosaccharides are considered soluble dietary fibre which shows prebiotic effects in the human digestion system (Mohd Nor *et al*., 2017). Galactomannan is an oligosaccharide which shows beneficial effects as a prebiotic compound (Majeed *et al*., 2018). In this respect, ADSP is a fraction of polysaccharide having the potential to use as food-grade fibre. Xyloglucan has proven to be effective in the treatment of acute diarrhoea and also has potential use in the pharmaceutical industry (Genesis *et al*., 2015; Kulkarni *et al*., 2017). Therefore, value addition to defatted coconut kernels by concentrating on polysaccharides will be a profitable industry.

Conclusion

Simple techniques were developed to concentrate soluble and insoluble polysaccharides from defatted desiccated coconut kernel. Trifluoracetic acid hydrolyzed coconut kernel soluble and insoluble polysaccharides into monosaccharides more effectively than sulphuric acid. The hydrolysis products indicated the presence of glucan, xyloglucan and galactomannan fragments in the hydrolysate and the coconut kernel polysaccharides developed in this study are likely to have application in food, feed and pharmaceutical industries. Further studies are needed to apply the findings in relevant industries.

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