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Physicochemical properties, *in-vitro* binding capacities for lard, cholesterol, bile acids and assessment of prebiotic potential of dietary fiber from cassava pulp

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Abstract

Cassava pulp is a high value by-product for dietary fiber production. Water-insoluble dietary fiber from cassava pulp (CDF) contained 79.03% (w/w) of neutral detergent fiber (NDF; cellulose, hemicellulose, lignin) and a high content of cellulose at 58.55% (w/w). Physicochemical properties of the CDF were studied. Moreover, the CDF was evaluated for its *in vitro* binding capacities for lard, cholesterol and bile acids: cholic acid (CA); deoxycholic acid (DCA); and taurocholic acid (TA) which were correlated to cholesterol-lowering properties. The digestive stability of CDF was 87.98%. The CDF showed a higher binding capacity for lard, cholesterol and bile acids compared to commercial cellulose. Binding with CA, DCA and TA were 38.50%, 42.71% and 40.84%, respectively. The CDF showed prebiotic activity for *Lactobacillus plantarum* TISTR 1465, which was higher than commercial cellulose, but lower than some soluble dietary fibers (Inulin, Lactulose and Fructooligosaccharide).

Introduction

It is well-known that dietary fibers act as a potential "functional food". The consumption of foods containing high fiber has many advantages for humans including the reduction of the incidence of cardiovascular diseases (CVDs) by reducing the risk of type-2 diabetes, body weight, and serum cholesterol levels, and by improving in the large bowel function (Bourdon *et al*., 2001; Merchant *et al*., 2003; Tucker and Thomas, 2009; Brownlee, 2011). Furthermore, dietary fiber can bind organic substances such as bile acids, toxic compounds and cholesterol in the small intestine, and increase the fecal excretion of these entities (Zhang *et al*., 2011; Mudgil and Barak, 2013). Because dietary fiber is not digested by the digestive system in the human body, it can be used as a non-caloric ingredient for the replacement of caloric ingredients, such as fats, carbohydrates and protein in many food products. Consequently, the consumption rate of fiber-rich products has increased. Many by-products from the fruit and vegetable industry are of particular interest due to their low cost and availability in large quantities. Indeed, some of the agricultural by-products, such as apples, citrus fruits, grapes, carrots and Brassica vegetables have already been used in the production of dietary fiber (Grigelmo-Miguel and Martín-Belloso, 1999; Figuerola *et al*., 2005; Hsu *et al*., 2006). With regard to the food industry, dietary fiber can also incorporate some functional properties of foods, such as increasing water and oil holding capacity, emulsification, and gel formation. Thus, they can be incorporated into many varieties of food products, such as bakery products, dairy products, jams, meats and soups etc. (Elleuch *et al*., 2011). However, various methods and different sources for obtaining dietary fiber might alter their chemical composition and physicochemical properties that would subsequently affect their function as food ingredients in food applications (Chau and Huang, 2003).

The term 'prebiotic' is defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth or the activity of one or a number of bacteria in the colon, that has the potential to improve health" (Gibson and Roberfroid, 1995). Prebiotics are often referred to as non-digestible oligosaccharides which are extracted from natural sources or synthesized from oligosaccharides.

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The purpose of prebiotic consumption is to increase the number of selected bacteria for production of various short-chain fatty acids (SCFAs; acetate, butyrate and propionate) because of their impact on the lower gut environment, metabolism, and disease prevention (Jenkins *et al*., 1999; Farnworth, 2007). The SCFAs are quickly absorbed and can serve as an energy source for the hosts, especially between meals. Moreover, the generation of SCFAs during fermentation of the prebiotic by the microflora in the gut suggests that this is one of the important mechanisms responsible for their lipid-lowering effects (Lovegrove and Jackson, 2001).

Cassava (*Manihot esculenta* Crantz) is the third-largest source of food carbohydrates in the tropics. Cassava is mostly grown in tropical countries. Nigeria is the world's largest cassava producer, followed by Brazil and Thailand. However, Thailand has a higher yield per area than Nigeria and Brazil. Thailand is currently one of the world's biggest exporters of cassava products. Cassava is planted in all regions of Thailand except the South. In 2015/16, more than 50% was planted in the Northeast of which the main province is Nakhon Ratchasima (Thai Tapioca Starch Association, 2016). Fifty percent of this is used as raw material for the production of tapioca or cassava starch and other byproducts such as cassava chips, cassava pellets and cassava flour. Cassava starch production is one of the most important agro-industries in Thailand. Thailand is the third largest producer of cassava starch, which produces 2.9 million tons per year and has a value of around 41.2 billion baht (Centre of Agricultural Information and Office of Agricultural Economics, 2016). Consequently, there are a lot of by-products from the processing of cassava of which the most important is cassava pulp. At least 1 million tons of pulp is generated annually in Thailand Cassava stem, soil, sand, and pulp are solid wastes from cassava starch production. Cassava pulp is the main by-product from the production process and results in creation around 10-15% of the original root weight (Figure 2.2). For starch production, 10 million tons of fresh roots can be generate at least 1 million tons of pulp annually (Sriroth *et al*., 2000). Nowadays, the wastes were use as soil additive. Pulp is fine and white with moisture content up to 75%. Carbohydrate, 55-56%, is the main composition. Starch remaining in the pulp may approximately be 50-60% of its dry weight, in which the starch mostly be trapped inside ligno-cellulose that refer to cellulose, hemicellulose and lignin. The pulp also contains pectin, cellulose, and fiber of approximately 10-15%, protein of 1.4-5%, fat of 0.1-5%. Other components are minerals: i.e., Fe^{2+} of 155 ppm, Mn^{2+} of 40 ppm, Mg^{2+} of 1100 ppm, Cu^{2+} of 4 ppm, and Zn^{2+} of 21 ppm per kg-dry pulp. Therefore, the aims of this study were to determine the physicochemical properties and binding capacity for lard, cholesterol, bile acids including the potential to be a prebiotic for lactic acid bacteria of dietary fiber from cassava pulp.

Materials and methods

Materials and sample preparation

Cassava pulp was obtained from Sanguan Wongse Industries Co., Ltd. in the local area of Nakhon Ratchasima province, Thailand. Heat-stable α-amylase (EC 3.2.1.1, Merk, Darmstadt, Germany), amyloglucosidase AMG 300L from *Aspergillus niger* (EC 3.2.1.3, Bray, Co. Wicklow, Ireland), neutrase® (EC 3.4.24.28 from Bacillus amyloliquefaciens, Novozymes Co., Bagsvaerd, Denmark) and commercial cellulose (HiMedia® Laboratories Pvt Ltd., Mumbai, India) were used. All chemicals used were of reagent grade.

Cassava pulp was dried at 60ºC in a tray dryer (Kluaynumtaitowop, Bangkok, Thailand) overnight. Before use, the dried cassava pulp was finely ground (GmbH & Co.KG D-42781, Haan, Germany) and stored at room temperature in a vacuum-packed container.

Dietary fiber preparation

Dietary fiber from cassava pulp (CDF) was prepared following the method described by Kachenpukdee *et al*. (2016). Cassava pulp solution was prepared at 4% (w/v) with phosphate buffer (50 mM, pH 6). Heat-stable α-amylase Termamyl 120 L (0.1% w/v) was added and heated for 30 minutes in a boiling water bath (with gentle shaking at 5 min intervals). After cooling to room temperature, the pH was adjusted to 7.5 with 0.17 M sodium hydroxide solution (NaOH) prior to adding neutrase (1% v/v). The neutrase was treated for 30 min at 60°C with continuous shaking. After cooling down to room temperature, the pH was adjusted to 4.5 with 0.205 M phosphoric acid solution (Carlo). Subsequently, 0.1% (v/v) amyloglucosidase was added to the mixture and treated for 30 min at 60°C with continuous shaking. The resulting hydrolysate was separated by centrifugation (Hettich, Universal 32R, DJB Labcare Ltd.) at 10000 x g for 10 min to separate the supernatant from the fiber-enriched sediment. The sediment was washed three times with distilled water, centrifuged again, dried at 60°C in a hot air oven and then finely ground. The dietary fiber powder was kept in a sealed container at 4°C until use.

Physicochemical and Functional Properties of Dietary fiber

The physicochemical property analyses, including crude protein, moisture, ash, fat, crude fiber, carbohydrate, acid detergent fiber (ADF) and acid detergent lignin (ADL), were performed according to the AOAC methods 979.10 (AOAC, 2005). Neutral detergent fiber (NDF) was assessed according to the methods of Van Soest *et al*. (1991). An analysis of functional properties included water holding capacity (WHC), water retention capacity (WRC), swelling capacity (SWC) (Robertson *et al*., 2000) and oil holding capacity (OHC) (Caprez *et al*., 1986). Starch content was analyzed by the Anthrone method (Hansen and Moller, 1975) with modification.

A monosaccharide analysis was also carried out. The sample was first hydrolyzed by 72% H_2SO_4 at 30ºC for 1 h. The residue was hydrolyzed with 1 M H_2SO_4 at 100ºC for 4 h, to give constituent monosaccharides. The monosaccharide hydrolysate was cooled, diluted with water, and filtered through a 0.45 µm filter before injection (Wood *et al*., 1994). A volume of 25 µl was injected into the High Performance Anion Exchange Chromatography (HPAEC) System (DIONEX) (Archemica international, Co. Ltd., Sunnyvale, CA, USA) and differentiated by a CaboPac PA1 column connected with Pulsed Amperometric Detection (PAD). Gradient elution was carried out using 250 mM NaOH and deionized water at a flow rate of 0.2 mL/min, with 20/100 to 0/100 at a linear gradient. The monosaccharide content was quantitatively analyzed by comparison with known individual standard curves (rhamnose, arabinose, galactose, glucose, xylose and mannose) at concentrations varying from 10 to 100 ppm.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Dried cassava pulp, CDF and commercial cellulose powder were dried and stored in desiccators prior to a FTIR analysis. FTIR spectra were recorded using a golden-gate diamond single reflectance ATR on a Bruker T27/Hyp 2000 FTIR spectrophotometer (Germany). The spectra were recorded in the transmittance mode from 4000 to 400 cm^{-1} (mid-infrared region) at a resolution of 4 cm^{-1} and 64 scans were collected. At least triplicate spectra readings for each sample were obtained.

Scanning Electron Microscope (SEM)

Samples were spread on a double side conducting adhesive tape, pasted on a metallic stub, coated with gold in a sputter coating unit for 8 min and observed in a JEOL JSM-6010LV electron microscope (JEOL ltd., Tokyo, Japan).

In vitro digestion for determination of digestive stability

Simulated digestion of CDF was performed according to Garrett *et al*. (1999) and Ferruzzi *et al*. (2001) with modification. The *in vitro* digestion consisted of simulated gastric and small intestinal phases of digestion.

Gastric phase

Briefly, 1 g of dried sample was homogenized with 20 mL of 120 mM NaCl in 50 mL polypropylene tube by homogenizer (model T25D, Germany), then the pH was adjusted to 2.0 ± 0.1 with 1 M HCl and 2 mL of porcine pepsin (40 mg/mL in 100 mM HCl) added. Then the volume was adjusted to 40 mL with 120 mM NaCl, filled with nitrogen gas, tightly capped and sealed with parafilm and incubated in a shaking water bath (JULABO, SW22, USA) for 1 h at 37ºC, 150 rpm.

Small intestinal phase

At the end of the gastric phase, the small intestinal phase was followed by raising the pH to 6.0 ± 0.1 with 1 M sodium bicarbonate (NaHCO3). A reaction tube was added with 3mL of crude bile extract (40 mg/mL in 100 mM NaHCO3) and 2 mL of pancreatin (12 mg/mL in 100 mM NaHCO3). Next, the pH of the sample was increased to 7.0 ± 0.1 with 1 M NaHCO3. Then, the tube was filled with nitrogen gas tightly capped and sealed with parafilm before being placed in a shaking water bath (JULABO, SW22, USA) for 2 h at 37ºC, and at 150 rpm to complete the intestinal phase of the *in vitro* digestion process. The mixture was centrifuged which induced precipitation and the sediment was analyzed for neutral detergent fiber (NDF).

$$\text{Digestive stability (\%)} = \frac{\text{Amount of NDF in digesta}}{\text{Amount of NDF in pre-digested sample}} \times 100$$

Binding capacity of dietary fiber for bile acids

The bile acids binding capacity was determined by colorimetry according to the method of Kahlon and Woodruff (2003) and Dziedzic *et al*. (2012) with minor modifications. The factor triggering the color reaction in this method is a 5% aqueous solution of furfural (Sigma-Aldrich®, MO, USA). Three bile acids were selected for analysis, i.e. cholic acid (CA, AMRESCO, Ohio, USA), deoxycholic acid (DCA, ACROS Organics, New Jersey, USA) and taurocholic acid (TA, ACROS Organics, New Jersey, USA) at a concentration of 1 mM. The principle of measurement was to determine concentrations of bile acids in the supernatant after incubation at a temperature of

37°C and the pH of the gastric juice. The analytical sample of CA, DCA and TA was dissolved in 25 mL of absolute ethanol, using ultrasounds, and then made up with phosphate buffer of pH 6.9. The sample of 0.5 g and 20 mL bile acids dissolved in the phosphate buffer was placed in a flask and incubated in a shaking water bath at a temperature of 37°C for 2 h. Additionally 0.5 g of the analysed material in phosphate buffer without bile acids was used for a blank. After incubation in a water bath at 37°C for 2 h, the sample was filtered. The supernatant was kept for further analysis.

The amount of 5 mL supernatant was mixed with 5 mL of 70% H_2SO_4 and left to stand at room temperature for 2 min. Then, 1 mL of 5% furfural solution was added (after 5 min pink coloring appeared), and the absorbance was measured. The amount of bile acids absorbed by dietary fiber was determined based on the difference in concentrations before and after incubation. The concentration of bile acids in the tested sample was determined based on the standard curve for a given acid within the range of concentrations which was 0.1 to 1 mM. Absorbance was measured using a spectrophotometer (Biochrom Libra S22 S/N 97765, UK) at a wavelength of 510 nm.

Calculation of bile acid binding capacity of dietary fiber (BA):

$$BA\ (\%) = \left(1 - \frac{\text{(Bile acid concentration after incubation)}}{\text{(Bile acid concentration before incubation)}}\right) \times 100$$

Binding capacity of dietary fiber for lard

The binding capacity of dietary fiber for lard was determined according to the method proposed by Zhang *et al*. (2011) with slightly modified. Dietary fiber (3 g) was mixed with melted lard (prepared from fresh pork) in a centrifugal tube and left undisturbed for 1 h at 37ºC. Then, the mixture was centrifuged at 10000×g for 10 min. The supernatant was decanted, and the pellet was recovered by filtration. The binding capacity (BOC) was calculated as follows:

$$BOC = (W_2 - W_1)/W_1$$

Where W_1 and W_2 are the weights of the dietary fiber before and after adsorbing lard, respectively.

Binding capacity of dietary fiber for cholesterol in egg yolk

In this research, egg yolk was used as a model system for determining the binding capacity of cholesterol since cholesterol is difficult to dissolve in water even after the addition of emulsifiers. The binding capacity of dietary fiber for cholesterol in egg yolk was determined according to the method described by Zhang *et al*. (2011) with slight modifications. Fresh egg yolk was diluted with 9 times the volume of deionized water. The pH of the mixtures of 2.0 g dietary fiber in 50 mL of the diluted yolk was adjusted to 2.0 and 7.0, respectively (similar to the pH conditions prevailing in the stomach and small intestine, respectively), then the mixture was shaken at 120 rpm in a water-bath at 37ºC for 2 h, and diluted yolk without dietary fiber added was used as a blank. At the end of adsorption, 4 mL of the sample was collected, and 16 mL of absolute ethanol was added to precipitate the sedimentation of the dietary fibers and they were centrifuged at 10000×g for 20 min. The ethanol in the supernatant was removed with a vacuum rotary evaporator (Buchi Rotavapor R-114, USA). One mL of the concentrate was diluted with 5 mL of 90% acetic acid. Color was developed according to the method of Park (1999) by adding 0.1 mL of o-phthaladehyde reagent and 2 mL of concentrated H_2SO_4. The mixture was left to stand for 20 min and the absorbance was measured at 550 nm against a reagent blank. The cholesterol concentration in the samples was determined against a standard curve generated by standard cholesterol (Sigma Aldrich®, Saint Louis, MO, USA) within the range of concentrations at 0.025, 0.050, 0.075, 0.100 and 0.125 mg/mL. The binding capacity (BC) was calculated as follows:

$$BC = [C_{blank} - (C_{supernatant}) \times F] \times (50/w)$$

Where C_{blank} and $C_{supernatant}$ are the cholesterol concentrations in the yolk without dietary fiber and the supernatant mixed with dietary fiber, respectively; F is the dilution factor; 50 is the adsorption volume (mL); and w is the weight of the dietary fiber.

Determination of prebiotic activity

Prebiotic activity was tested following the method described by Huebner *et al*. (2008). Five *Lactobacillus* strains (3C2-10, 21C2-10, 21C2-12 and OC4-4) from Nawong (2015) and *Lactobacillus plantarum* TISTR 1465 (Thailand Institute of Scientific and Technological Research) were cultured in MRS-broth with 2% prebiotics. Cassava pulp dietary fiber (CDF), Fructooligosaccharide (FOS; Sigma Aldrich®, Saint Louis, MO, USA), Lactulose (LAC; ACROS Organics™, Thermo Fisher Scientific Inc., New Jersey, USA), Inulin (Sigma Aldrich®, Saint Louis, MO, USA) and Cellulose (HiMedia®

Laboratories Pvt. Ltd., Mumbai, India) were tested and incubated at 37°C under anaerobic conditions for 24 h. All samples were already passed the *in vitro* digestion process before the determination of the prebiotic activity. The prebiotic activity score was determined using the following equation:

$$\text{Prebiotic activity score} = \left[\frac{(\text{probiotic log CFU on the prebiotic at 24 h} - \text{probiotic log CFU on the prebiotic at 0 h})}{\text{probiotic log CFU on the glucose at 24 h} - \text{probiotic log CFU on the glucose at 0 h}}\right] - \left[\frac{(\text{enteric log CFU on the prebiotic at 24 h} - \text{enteric log CFU on the prebiotic at 0 h})}{\text{enteric log CFU on the glucose at 24 h} - \text{enteric log CFU on the glucose at 0 h}}\right]$$

Statistical analysis

All experiments were performed in triplicate and mean values (on dry basis) with standard deviations are reported. The experimental data were analyzed using an analysis of variance (ANOVA). SPSS® software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical calculations.

Results and discussion

Chemical compositions of cassava pulp and dietary fiber from cassava pulp (CDF)

The proximate analysis of cassava pulp powder and CDF are shown in Table 1. The pulp still contained high starch contents (58.11%) while CDF contained high cellulose (58.55%) in an insoluble form. There was a high starch content of more than 50% in the pulp because the starch granules, which are called "bound starch", are bound inside a complex structure of the pulp which still remains and is difficult to separate after the rasping step (Saengchan *et al*., 2015). Many studies reported that cassava pulp is composed mainly of starch followed by cellulose, hemicellulose and lignin, respectively (Suksombat *et al*., 2007; Kosoom *et al*., 2009; Rattanachomsri *et al*., 2009; Ali *et al*., 2011). Cellulose content in the pulp was higher than that shown by other food industry residues such as malt bagasse, oat hull, rice hull and fibrous residue of banana pseudo-stems, but lower in hemicellulose and lignin content (Jacometti *et al*., 2015). Therefore, cassava pulp can possibly be used in the preparation of dietary fiber. The results showed that CDF had a higher content of NDF, ADF, ADL, cellulose and hemicellulose when it passed through the extraction process due to the hydrolysis properties of the enzymes used. The removal of starch is necessary for dietary fiber preparation. Thus, thermostable α-amylase was subjected to high temperature in the cassava pulp solution to release the trapped starch granules. This enzyme hydrolyzes α-1,4 glyosidic bonds of starch by random cleavage, but does not hydrolyze α-1, 6 glycosidic bonds of amylopectin in the starch granules. Amyloglucosidase was used to hydrolyze α-1,4 glycosidic bonds from the non-reducing end of starch. This enzyme can hydrolyze α-1,6 glycosidic bonds of amylopectin at a slow rate (Pandey *et al*., 2000). Meanwhile protein in the raw material was eliminated by neutrase.

The NDF definition indicated that CDF had a high content of insoluble dietary fiber, which was cellulose, hemicellulose and lignin. The beneficial technological functionality and physiological effects of insoluble fibers are characterized by their porosity, low density and ability to increase fecal bulk and reduce intestinal transit time (Elleuch *et al*., 2011). While the physiological effects of soluble fibers reduce serum LDL cholesterol levels and improve glucose metabolism and insulin response (Glore *et al*., 1994). However, some recent research illustrates that insoluble fibers isolated from some fruits, vegetables, and pomace can also effectively decrease serum cholesterol and triglyceride (Chau and Cheung, 1999; ErkkilaÄ *et al*., 1999; Knopp *et al*., 1999; Rosamond, 2002; Chau *et al*., 2004). The ability of cholesterol and bile acid absorption and the ability to enhance excretion via feces are important properties of water-insoluble fibers leading to lower cholesterol concentration. Thus, CDF could possibly be used as an ingredient to lower blood cholesterol levels.

The sugar profile of cassava pulp and CDF showed that glucose was the most abundant constituent (Table 1). Chaikaew *et al*. (2012) indicated that the main monosaccharide of cassava pulp is glucose followed by galacturonic acid, xylose, galactose, arabinose, mannose and rhamnose. Rattanachomsri *et al*. (2009) also reported that monosaccharides of cassava pulp is composed mainly of glucose, followed by galacturonic acid, xylose, galactose, arabinose, mannose, and rhamnose. In CDF, glucose was present in the highest amounts followed by galactose, rhamnose, xylose, arabinose and mannose, respectively. The results revealed that the glucose content of CDF clearly decreased when compared with the raw material. This might be due to the reduction of starch content and, secondly, to the effects of enzyme activity. Other increases in monosaccharides are correlated to increases of hemicellulose and lignin content (Table 1) of which the main chain of hemicellulose is arabinose and xylose (Mongeau and Brooks, 2016).

Table 1. Chemical compositions (%) of cassava pulp powder and dietary fiber from cassava pulp (CDF) (dry weight basis).

Components	Cassava pulp (%)	CDF (%)
Crude protein	2.02 ± 0.19	1.01 ± 0.10
Fat	0.21 ± 0.08	0.25 ± 0.06
Moisture	6.63 ± 0.11	5.52 ± 0.09
Ash	3.76 ± 0.05	4 .52 ± 0.04
Crude fiber	17.23 ± 0.13	40.24 ± 2.22
Carbohydrate	70.15	48.46
Starch	58.11 ± 0.06	8.50 ± 0.31
Neutral detergent fiber (NDF)	31.40 ± 0.58	79.03 ± 0.51
Acid detergent fiber (ADF)	25.08 ± 0.17	70.14 ± 0.40
Acid detergent lignin (ADL)	4.16 ± 0.10	11.59 ± 0.01
Cellulose[a]	20.92	58.55
Hemicellulose[b]	6.30	8.89
Monosaccharide (relative %)		
Galactose	6.95 ± 0.02	14.90 ± 0.10
Glucose	76.66 ± 0.40	48.85 ± 0.38
Xylose	2.34 ± 0.13	10.56 ± 0.06
Mannose	0.64 ± 0.11	2.81 ± 0.12
Rhamnose	7.92 ± 0.08	12.89 ± 0.16
Arabinose	5.49 ± 0.07	9.99 ± 0.12

[a]ADF-ADL, [b]NDF-ADF

Functional properties of CDF

The functional properties of CDF compared with cellulose, which is the most used for food fiber, are itemized in Table 2. The results show the values for swelling capacity (SWC), water retention capacity (WRC), water holding capacity (WHC) and oil holding capacity (OHC) of CDF were 4.82 mL/g, 8.36 g/g dry weight, 8.17 g/g and 3.97 g/g, respectively. The hydration properties of dietary fiber refer to WHC, SWC and WRC which are correlated to the chemical structure of the component polysaccharides, and other factors such as particle size, porosity, ionic form, pH, temperature, ionic strength, type of ions in solution and stresses upon fibers. The ability of dietary fibers to hold water is strongly related to the source of the dietary fiber (Elleuch *et al*., 2011). Furthermore, hydration properties are correlated with both physiological and technological aspects and can influence the incorporation of fiber-enriched ingredients into food products (Femenia *et al*., 1999). The WHC represents the volume of a hydrated sample under centrifugal force, while SWC represents the volume of a hydrated sample under gravity forces (López *et al*., 1996). Dietary fibers with high WHC can be used as functional ingredients to avoid syneresis and they modify the viscosity and texture of some formulated foods (Grigelmo-Miguel and Martín-Belloso, 1999). The results obtained for WHC, SWC and WRC were higher than those for cellulose. Moreover, the WHC of CDF was higher than that of onion by-products, malt bagasse, oat hull, rice hull and fibrous residue from banana pseudo-stems, passion fruit seeds, but lower than Polygonatum odoratum, the peel of citrus fruit and peach pulp and peel (Chau and Huang, 2003, 2004; Benítez *et al*., 2011; de Escalada Pla *et al*., 2012; Lan *et al*., 2012; Jacometti *et al*., 2015). Insoluble dietary fibers which have a high WHC can increase fecal bulk and reduce the gastrointestinal transit time, which may be linked to the prevention and treatment of different intestinal disorders, including constipation, diverticulitis, haemorrhoids and other bowel conditions (Goñi and Martin-Carrón, 1998). Regarding SWC, the result obtained was similar to that for malt bagasse, oat hull, rice hull, fibrous residue from banana pseudo-stem, pea and chickpeas (Tosh and Yada, 2010; Jacometti *et al*., 2015). The main factors effecting SWC and WHC are particle size, polysaccharide composition and the intermolecular organization of plant cell walls (López *et al*., 1996; Serena and Knudsen, 2007). WRC is defined as the quantity of water that remains bound to the hydrated fiber when subjected to an external force such as pressure of centrifugation (Ma and Mu, 2016). The WRC obtained was higher than that of deoiled cumin, grapefruits, citrus fruits, apples and bananas (Figuerola *et al*., 2005; Ma and Mu, 2016).

The result obtained for OHC was similar to that of maca residue, although higher than that for peel and pulp of peach, soybean DF and some orange DF products (Grigelmo-Miguel and Martín-Belloso, 1999; de Escalada Pla *et al*., 2012; Chen *et al*., 2015). The differences in OHC among various dietary fibers might be attributed to their chemical and physical differences (Chau and Huang, 2003). The above results showed high levels of WHC, SWC, WRC and OHC of CDF which have potential use as food ingredients to reduce caloric levels in the food industry.

Some authors illustrate that the technological properties of dietary fiber products (WHC, SWC, WRC and OHC) affect their physiological functionality (Jenkins *et al*., 2004; Elleuch *et al*., 2011). In addition, some properties of fiber preparations such as WHC, SWC and OHC have significant characteristics which make them suitable for food additives. These characteristics can be adjusted by mechanical, chemical, and thermal processes (Elleuch *et al*., 2011).

Table 2. Functional properties of dietary fiber from cassava pulp (CDF) and commercial cellulose.

Functional properties	CDF	Commercial cellulose
Water holding capacity (g/g dry weight)	8.17 ± 0.40	4.92 ± 0.33
Water retention capacity (g/g dry weight)	8.36 ± 0.20	5.95 ± 0.49
Swelling capacity (mL/g)	4.82 ± 0.15	1.13 ± 0.15
Oil holding capacity (g/g)	3.97 ± 0.14	2.66 ± 0.09

Fourier Transform Infrared Spectroscopy

The FTIR spectrum of cassava pulp, CDF and commercial cellulose are shown in Figure 1. The FTIR spectrum of carbohydrates is used for the identification of its chemical structure. Normally, the spectrum at wave numbers between 1200 and 950 cm^{-1} is called the molecular fingerprint of which the position and intensity of the bands are specific for each major chemical group in polysaccharides (Černá *et al*., 2003). The FTIR spectrum of CDF exhibited similarities to the absorption pattern for the raw material. When compared with cellulose, the CDF spectrum was more similar to cellulose than cassava pulp, confirming the preliminary result from the chemical composition analysis (Table 1) that the most important component in CDF was cellulose which is insoluble. Peaks at 895 cm^{-1} are indicative of stretching vibrations of β-glycosidic linkages in polysaccharides (Ma and Mu, 2016). The sharp peak appearing at around 1028, 1020 and 1003 cm^{-1} is indicative of the stretching vibrations of pyranose (Ying *et al*., 2011). The band at 1240 cm^{-1} of CDF indicates the presence of acetyl group substitution of some of the –OH groups present (Mathlouthi and Koenig, 1986). The bands at 1370 cm^{-1} point to ring breathing with C–H stretching. Both cassava pulp and CDF are composed of protein which usually has specific absorption bands in the 1700-1500 cm^{-1} region. The carbonyl (C=O) stretching was at 1735 and 1605 cm^{-1}. A shoulder peak at 1735 and 1730 cm^{-1} was found in both the CDF and the cassava pulp, but it was not found in cellulose. This peak could indicate hemicellulose (Himmelsbach *et al*., 2002). The bands at 3000-2800 cm^{-1} are indeed characteristic of C-H vibrations from some methylene groups of polysaccharides (Ma and Mu, 2016) and can be associated with the ring hydrogen atoms in lignocellulosic components such as cellulose, hemicellulose and lignin (Ibrahim *et al*., 2011). There is an intense peak at 3331 and 3317 cm^{-1} which can be attributed to O–H stretching of the hydrogen bound to the hydroxyl groups originating mainly from cellulose, hemicellulose and lignin (Jacometti *et al*., 2015; Ma and Mu, 2016).

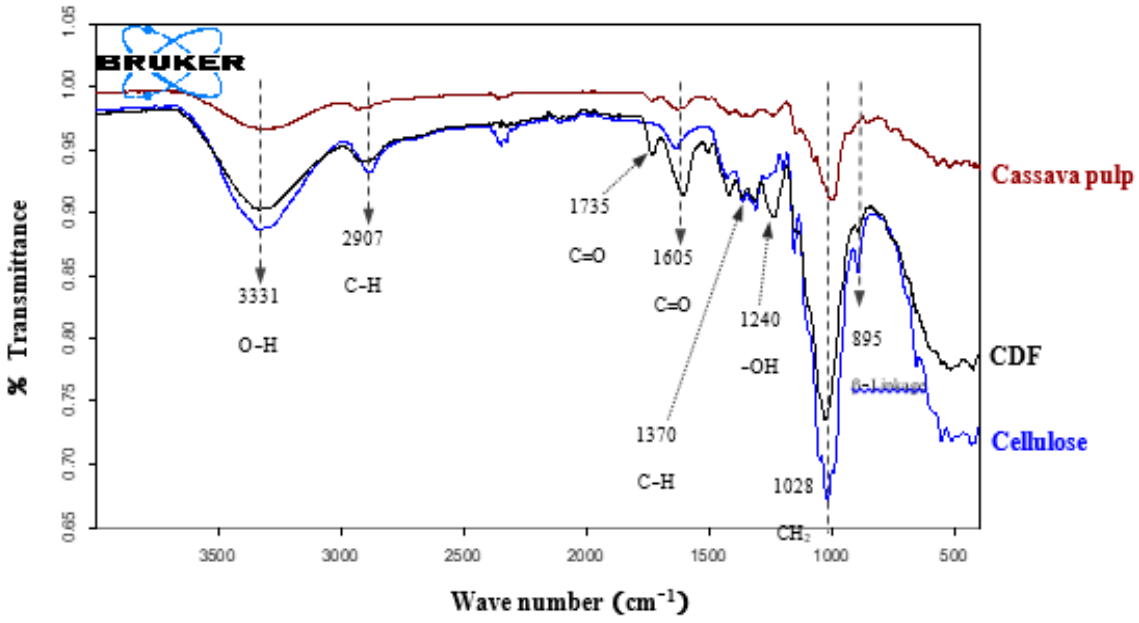

Figure 1. Fourier transform infrared spectrum of cassava pulp, dietary fiber from cassava pulp (CDF) and commercial cellulose.

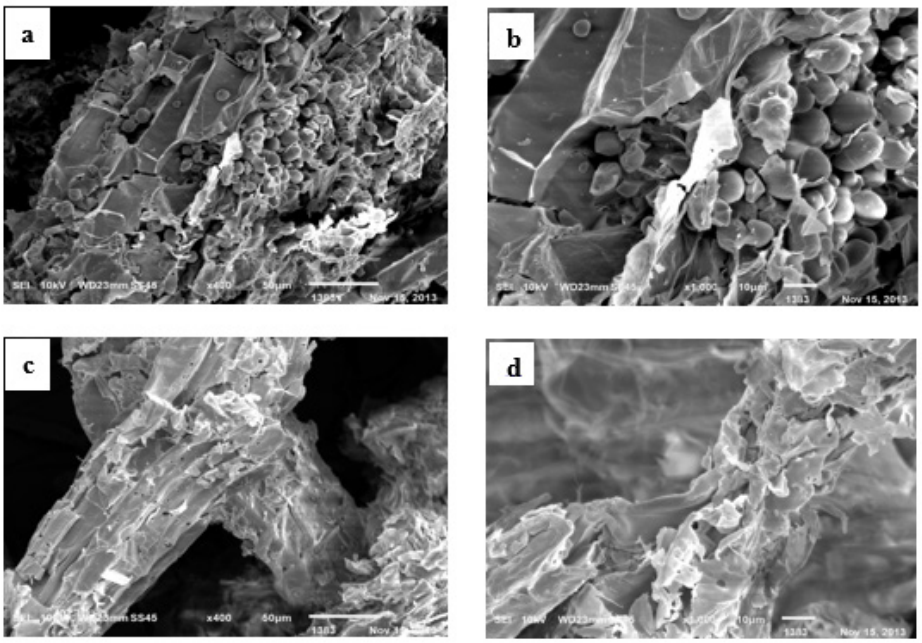

Figure 2. Scanning electron micrographs of cassava pulp (a & b) and CDF (c & d) at 400 and 1000x.

Scanning electron microscopy study

From the SEM results, the pulp shows huge starch granules embedded well inside the matrix which is indicative of starch content as mentioned above (Figure 2a, b). With regard to CDF, after hydrolysis by enzymes, the SEM results show that there are no starch granules embedded inside the fiber matrix (Figure 2c, d) and, although starch still remains at about 8.50% (Table 1), it might be due to an over estimation of the starch determination method (Anthrone method). However, this CDF shows higher purification from starch and its fiber structure seems to have high porosity. The CDF tends to be decontaminated by starch granules.

Determination of digestive stability

Digestive stability is defined as the percentage of NDF (cellulose, hemicellulose and lignin) of CDF recovered in the precipitate. The digestive stability of CDF was 87.98%. This value seems high because CDF is composed of high amounts of cellulose, hemicellulose and lignin which are insoluble and resistant to human enzymes (Mongeau and Brooks, 2016).

Binding capacity of dietary fiber for bile acids

The bile acid binding capacity of dietary fiber is related to cholesterol-lowering properties for the following reason. Normally, bile acids are synthesized in the liver from cholesterol and secreted into the small intestine to allow digestion of dietary fats and oils by acting as a surfactant. When dietary fiber binds bile acids in the small intestine, the fiber-bile acid complex prevents bile acids from being reabsorbed from the small intestine, enhancing the secretion of bile acids. To replace the lost acids, cholesterol is drawn from the circulation to produce bile acids, thereby reducing the blood cholesterol levels. Therefore, the ability of bile acid absorption, which is able to enhance the excretion via feces, is one of the main mechanisms for cholesterol-lowering effects by other digestion-resistant polysaccharides (Gallaher *et al*., 2000; Zhou *et al*., 2006; Zacherl *et al*., 2011). *In vitro* studies have been widely used to predict the potential bile acid-binding capacity of many polysaccharides including chitosan, wheat bran, soy bean hull, apple peel, citrus fruits and other dietary fibers (Zhou *et al*., 2006; Zhang *et al*., 2011; Wang *et al*., 2015). CDF was studied for its direct binding capacity against bile acid including cholic acid, deoxycholic acid and taurocholic acid. Cholic acid-primary bile acid and deoxycholic acid-secondary bile acid were used as the representative of the bile acid binding capacity of dietary fiber. While taurocholic acid was chosen because of its higher UV activity although both deoxycholic acid and lithocholic acid which are secondary bile acids, seem to be appropriate standards for the determination of bile acid binding (Cornfine *et al*., 2010). In this experiment, commercial cellulose was used as a reference for low bile acid binding capacity (van Bennekum *et al*., 2005; Dongowski, 2007; Cornfine *et al*., 2010). The CDF showed that the *in vitro* bile acid binding capacities for CA, DCA and TA were 38.50%, 42.71% and 40.84%, respectively. The CDF showed a higher bile acid binding capacity compared

to commercial cellulose (12.33%, 15.90% and 13.37%, respectively) which may be due to chemical composition, especially for lignin. The major physical properties of lignin are that it can absorb bile acid and delay nutrient adsorption in the small intestine (Prosky and DeVries, 1992). Other studies have reported similar bile acid binding capacities for lupins product, grape fruit, buckwheat hull and buckwheat bran (Cornfine *et al*., 2010; Dziedzic *et al*., 2012; Wang *et al*., 2015). However, some studies exhibited lower bile acid binding capacities for insoluble dietary fiber preparations from soy beans or black eye beans, which could be due to differences in the *in vitro* bile acid binding assay, the different raw materials, or the composition and structure of the plant cell wall materials (Kahlon and Shao, 2004; Cornfine *et al*., 2010).

Binding capacity of dietary fiber for lard and cholesterol

The binding capacity for lard of CDF and commercial cellulose were 3.85 and 1.92 g/g, respectively. Whereas the binding capacity for cholesterol of CDF at pH 2 and 7 were 5.27 and 7.16 mg/g respectively; which was higher than commercial cellulose at 1.55 and 2.94 mg/g respectively. The results show that CDF had a higher binding capacity for lard and cholesterol than for commercial cellulose. This may be due to the fact that CDF is composed of cellulose, hemicellulose and lignin of which hemicellulose may be the main component promoting this effect. The important physical property of hemicellulose is its cation exchange capacity. Hemicelluloses can prevent cholesterol absorption by directly binding with cholesterol (Jalili *et al*., 2007; Mudgil and Barak, 2013). Thus, CDF shows a higher cholesterol binding capacity than commercial cellulose. The binding capacity for lard of CDF is similar to that of apple peel fiber (4.57 g/g) (Zhang *et al*., 2011). While the binding capacity for cholesterol of CDF is similar to that of soybean hull fiber, ponkan fiber and orange fiber (Zhang *et al*., 2011; Wang *et al*., 2015). This indicates that CDF has potential cholesterol-lowering properties.

Determination of prebiotic activity

Prebiotic activity scores of five *Lactobacillus* strains: 3C2-10, 21C2-10, 21C2-12, OC4-4, which were isolated from cassava pulp (Nawong, 2015) and *Lactobacillus plantarum* TISTR 1465 which is a probiotic strain (Thailand Institute of Scientific and Technological Research) are shown in Figure 3. Prebiotic activity of *Lactobacillus plantarum* TISTR 1465 of CDF shows a higher value than the prebiotic activity of commercial cellulose, but it is lower than some soluble dietary fibers. In addition, there was no prebiotic activity for the rest of the *Lactobacillus* strains tested. This result indicates that CDF has the potential to be a prebiotic source for some species of *Lactobacillus* strains. The main chemical components of CDF are high cellulose and hemicellulose. Although cellulose is not digested by human enzymes, it can be partially digested in the gut by beneficial microflora. Cellulose is degraded by natural fermentation in the colon to about 50% and produces a significant amount of short-chain fatty acids (SCFAs) which feed our intestinal cells. Moreover, microflora in the gut can digest hemicellulose which increases the number of beneficial bacteria and produces SCFAs which the colon cells use as fuel and they also affect cholesterol reduction (Jalili *et al*., 2007; Mudgil and Barak, 2013).

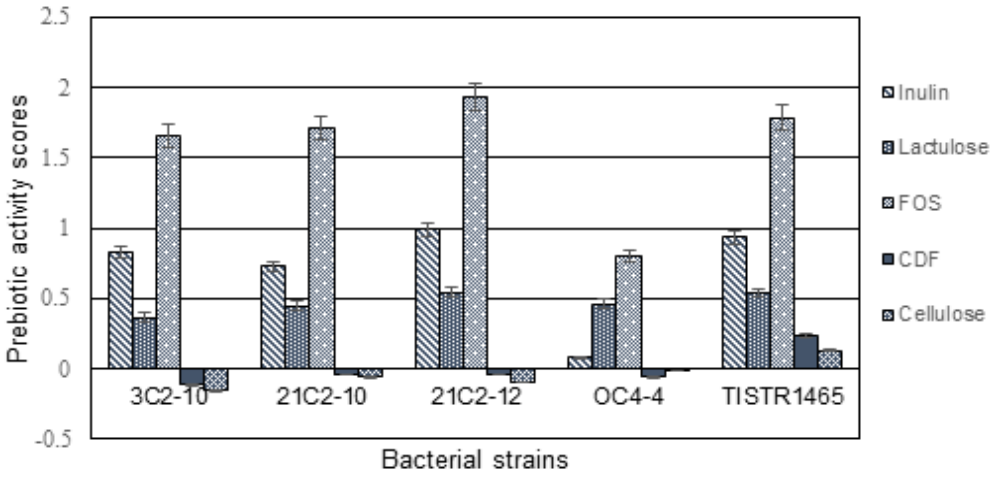

Figure 3. Prebiotic activity scores of Lactobacillus strains (3C2-10, 21C2-10, 21C2-12, OC4-4 and Lactobacillus plantarum TISTR 1465) of Inulin, Lactulose, Fructooligosaccharide (FOS), dietary fiber from cassava pulp (CDF) and commercial cellulose (cellulose).

Conclusions

Dietary fiber from cassava pulp has the potential to bind with lard, cholesterol and bile acid which is correlated to its cholesterol-lowering properties. Moreover, assessment of the prebiotic potential for some *Lactobacillus* strains leads to the conclusion that CDF has prebiotic activity for *Lactobacillus plantarum* TISTR 1465 and might also act as a prebiotic substance. An *in vivo* study of the cholesterol-lowering properties of CDF would be an interesting topic for further study.

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