Physicochemical, Functional and prebiotic properties of dietary fiber prepared from rice hull

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ABSTRACT: Rice hulls consist of 47 % crude fibers and 59.50% total dietary fibers (dry weight basis), which are main byproducts after the rice milling process, and can cause serious environmental waste disposal problems. However, rice hull is suggested as very high content of dietary fiber for extraction the value added products. Results showed that extraction yields of dietary fiber from rice hulls extracted with water, 4 M hydrochloric acid and 4 M sodium hydroxide at 70 ^{O}C for 10 h were 75.41, 26.42 and 23.94%, respectively. Chemical properties, including, total dietary fiber, holo-cellulose, alpha-cellulose and Klason lignin were reported. Color of lightness (L* value) of dietary fiber after extraction with hydrochloric acid and sodium hydroxide was significantly darker than extraction with water ($p \leq 0.05$). Functional properties (water holding capacity and oil holding capacity of dietary fiber) of the extract with sodium hydroxide were significantly higher ($p \leq 0.05$) at 9.90 g water/g powder and 8.89 g oil/g powder, respectively. Furthermore, dietary fibers extracted from rice hull were used as a carbon source for the cultivation of lactic acid bacteria (Lactobacilli and Bifidobacteria). It was found that the dietary fibers significantly stimulated the growth of two biotic strains ($p \leq 0.05$). Moreover, the sugar compositions of dietary fiber extracts were analyzed using high performance liquid chromatography (HPLC) with an evaporating light scattering detector (ELSD). Glucose and xylose were the main sugars found in dietary fibers extract. These results clearly indicate that dietary fiber preparation from rice hull is a valuable source of biopolymer which is potentially useful in food applications, especially in functional food products and probiotic substrates.

1. INTRODUCTION

Rice hulls are an agricultural residue made up of organic compounds, found abundantly in Thailand. Rice hulls are a major product of the rice milling process. Elimination of these residues by burning causes air pollution with toxic pollutants such as carbon monoxide, oxides of nitrogen, and volatile organic compounds (United States Environmental Protection Agency, 2012). However, the hull is mostly composed of dietary fiber components. In 2001 the American Association of Cereal Chemists or AACC submitted the definition of dietary fiber as "dietary fiber is the edible part of plant or analogous carbohydrate that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine". Moreover, dietary fiber includes oligosaccharides and associated plant substances for example cutin, wax, isoprene and lignin. Lignin is a water insoluble made up of 3-dimension polyphenolic acid polymer which decreases water permeability through the plant cell wall. Removing the lignin can improve chemical and functional properties of dietary fiber. Normally, lignin are divided into 2 types 1) acid soluble lignin and 2) acid insoluble lignin or "Klason lignin" (Technical Association of the Pulp and Paper Industry, 2006). Dietary fibers are generally classified into 2 types based on their water solubility i.e. water soluble (such as oligosaccharides, pectin, gum and carboxy methyl cellulose; CMC) and water insoluble (such as cellulose and hemicellulose) (Mudgil, 2013). However, oil holding capacity, water holding capacity and prebiotic property depend on the composition of fiber. Therefore, the objectives of this research were 1) to examine the effect of various solvents; water, hydrochloric acid, and sodium hydroxide on the physicochemical and functional properties of prepared dietary fiber, and 2) to study *in vitro* prebiotic properties of extracted dietary fiber from rice hull.

2. EXPERIMENT

2.1 Materials and Methods

Rice hull was obtained from Nattakriskarn Agritech Co., Ltd (Bangkok, Thailand). Rice hull was washed with water for cleaning and the wet rice hull dried by a hot air oven (70 ^{O}C) overnight. Dried rice hull was grinded and sieved through a 40 mesh size screen. The ground and unprocessed rice hulls were namely RH-C. The RH-C was chemically analyzed based on the Association of Official Analytical Chemists (AOAC) method (2000). After that, the amount of total dietary fiber (TDF) of the rice hull control was quantified using the AOAC 985.29 (2003). The levels of holo-cellulose (HC) and alpha-cellulose (AC) were analyzed by acid chlorite (Wise et al., 1946) and TAPPI T 203 om-88, respectively. Klason lignin (KL) contents were determined by gravimetric method (TAPPI T 222 om-88), as the amount of acid-insoluble material remaining after 72% H_2SO_4 hydrolysis. The color value of the RH-C was determined using colorimetry with CIE *L**, *a** and *b** color scale (Minolta model CR-10). Amount of functional properties i.e. water holding capacity (WHC) and oil holding capacity (OHC) were determined using the protocol described by Yang (2014) with slight modifications.

2.2 Extraction process

A 50 g sample of rice hull was treated with 1 Liter of solvent, at 70 ^{O}C for 10 h. After extraction, the hulls were washed with water until the pH of the fibers became neutral and were then dried by a hot air oven at 70 ^{O}C until dried. The rice hull process was namely RH-T. Amounts of TDF, HC, AC, KL, color value and functional properties of RS-T were determined as described above.

2.3 Sugar analysis

Analysis of sugar content was performed on a HPLC 1200 series Agilent technologies (USA) using a Zorbax carbohydrate (4.6 x 150 mm, 5 micrometer resin) column. Acetonitrile:water (75:25 v/v) was used as the mobile phase at 0.5 mL/min flow rate. Sugar calibration standards were glucose, xylose, fructose, arabinose, ribose, rhamnose and melibiose. All sugar standard solutions were prepared to cover the sugar concentration ranges of the samples tested.

2.4 Prebiotic properties

Dietary fiber obtained from the extraction process and reference prebiotics (oligosaccharides and inulin) were used as a carbon source for the growth of 2 strains of probiotics. Inoculum of *Lactobacillus casei* and *Bifidobacterium longum* were prepared by cultivation on MRS broth at 37 ^{O}C for 48 h. Nine milliliter of MRS broth with various carbon sources was prepared (2% w/v), then the inoculum (1 mL) was added to obtain a final concentration of 10^8 CFU/mL. The culture was incubated at 37 °C for 48 h in an anaerobic jar. The sample was taken after 48 h for bacteria numbering using the plate count method.

2.5 Statistical analysis

All treatments were carried out in triplicate. The results are expressed as mean ± S.D. (standard deviation). The SAS statistical computer package was used to analyze the experimental data (SAS User's Guide version6, 4th ed., SAS Institute, Cary, NC, USA, 1990). Significant differences ($p \leq 0.05$) between means were determined by Duncan's Multiple Range Test.

3. ANALYSIS

3.1 Proximate compositions

Proximate compositions of RH-C are shown in Table 1. Rice hull was an excellent fiber source for dietary fiber extraction because it contained crude fiber content greater than 47% (wet weight).

Table 1 Proximate analysis of rice hull control (RH-C) (wet basis).

Proximate analysis	% (Wet weight)
Moisture	5.89 ± 0.07^d
Protein	2.00 ± 0.01^e
Fat	0.42 ± 0.02^f
Crude Fiber	47.24 ± 0.10^a
Ash	14.64 ± 0.01^c
Carbohydrate	29.81 ± 0.06^b

a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.01$).

3.2 Physicochemical determination

After extraction, the visual colors of RH-T were changed (Fig. 1). The brightness *(L* value), a** and *b** of the RS-T were shown in Table 2. The *L* value* decreased directly with reaction time from about 53.80 to 30.20 in 10 h due to the result of Maillard reaction compounds (Lario et al., 2003). RH-T extracted with 4 M HCl was darker than RH-C due to 5-hydroxymethyl-2-furfuraldehyde (HMF) and 2-furaldehyde (2-FA) (Lario et al., 2003; Tosun, 2004; Hsieh et al., 2009) and RH-T extracted with 4 M NaOH was the darkest because the hydroxide anions (from NaOH) and phenol molecules (from flavonoids and Klason lignin) developed to brownish sodium phenoxide molecules.

a) b) c) d)

Fig. 1 Visual color change between a) RH-C b) RH-T by H_2O c) RH-T by 4 M HCl d) RH-T by 4 M NaOH

Table 2 The effect of extraction processing on color of dietary fibers.

Dietary Fibers	Solvents	Color		
		*L**	*a**	*b**
RH-C	-	53.80 ± 0.13^a	7.84 ± 0.04^d	24.90 ± 0.16^d
RH-T	H_2O	42.97 ± 0.06^b	10.11 ± 0.03^b	31.88 ± 0.09^a
	4 M HCl	34.38 ± 0.13^c	11.75 ± 0.14^a	29.52 ± 0.18^b
	4 M NaOH	30.20 ± 0.17^d	9.89 ± 0.07^c	28.60 ± 0.24^c

a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.01$).

3.3 Chemical properties and Functional properties determination

The result of the chemical analysis showed that RS-C contained about 59.50 % total dietary fiber, 53.98%

holo-cellulose, 55.95% alpha-cellulose and 32.17% Klason lignin (dry weight, Table 3). Total dietary fiber, holo-cellulose and alpha-cellulose content of the prepared dietary fiber directly increased with 4 M NaOH alkaline treatment from 59.50% to 79.42%, 53.98% to 78.66% and 55.95% to 95.75% (dry weight), respectively. The increase of total dietary fiber, holo-cellulose and alpha-cellulose might be a relative increase, caused by the reduction of other components such as Klason lignin. Klason lignin, which is the most insoluble component of dietary fiber decreased linearly with reaction time from 32.17% to 15.61% (dry weight) for 10 h with 4 M NaOH. Because the treatment of the lingocellulosic biomass with NaOH solution (pH 12-13) solubilized a portion of Klason lignin, about 50-60% present in the cell wall (Gould et al., 1989). Moreover, NaOH alkaline treatment resulted in disruption of the substrate morphological and an increase in the particles capacity to hold oil and water (Kerley et al., 1985). According to Fincher and Stone (1986) and Jung and Deetz (1993), Klason lignin was not significantly removed when extracted with water. Due to the fact that Klason lignin can form resistant linkages in the matrix of polysaccharides.

The results obtained for OHC, WHC are presented in Table 3. The varying solvents resulted in the levels of OHC and WHC contents. The highest of these was found in RH-T treatment in 4 M NaOH. OHC and WHC of dietary fiber from 4 M NaOH significantly increased with the reaction time from about 2.34 to 8.89 g oil/g dry fiber and 3.69 to 9.90 g water/g dry fiber within 10 h ($p \leq 0.05$), respectively. Because the alkaline solution solubilized a portion of lignin present in the cell wall, resulting in the disruption of the cell wall by hydrolyzing uronic, acetic esters and alpha-ether linkages between lignin and hemicellulose. The free hydroxyl group became available to bind to water molecules, thereby enhancing the OHC and WHC of the dietary fiber (Sangnark and Noomhorm, 2003).

Table 3 The effect of extraction processing on chemical properties and functional properties of dietary fibers.

Properties		Dietary Fibers			
		RH-C	RH-T		
			H_2O	4 M HCl	4 M NaOH
% Extraction Yield		100.00^{a}	75.41±1.05^{b}	26.42±1.48^{c}	23.94±0.50^{d}
Chemical Compositions (%)	TDF	59.50±0.04^{c}	59.42±1.11^{c}	72.73±2.62^{b}	79.42±0.08^{a}
	HC	53.98±0.04^{c}	50.44±1.35^{d}	63.51±1.44^{b}	78.66±0.32^{a}
	AC	55.95±0.05^{d}	59.79±0.76^{c}	83.19±3.52^{b}	95.75±0.07^{a}
	KL	32.17±0.26^{a}	31.52±1.73^{a}	23.50±0.16^{c}	15.61±0.18^{d}
Functional Properties	OHC^{x}	2.34±0.04^{d}	3.43±1.24^{c}	5.41±2.33^{b}	8.89±0.04^{a}
	WHC^{y}	3.69±0.02^{d}	4.95±0.93^{c}	5.91±1.92^{b}	9.90±0.14^{a}

^{x} (g oil/ g dry fiber)
^{y} (g water/ g dry fiber)
a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.01$).

3.5 Effect of extraction process (4 M NaOH) on sugar compositions analyzed by HPLC

It was found that 4 M NaOH had marked effects on the chemical compositions and functional properties of the RH-T obtained. Then, sugars of 4 M NaOH RH-T were analyzed by HPLC. The RH-T consisted mostly of glucose and xylose, as shown in Fig. 2. Glucose concentration of 4 M NaOH RH-T (0.5917 mg/ 1 mL) was significantly higher than xylose concentration (0.103 mg/ 1 mL), as shown in Table 4. Because the glucose polymer in RH-T is represented as cellulose and xylose is represented as hemicellulose in the dietary fiber.

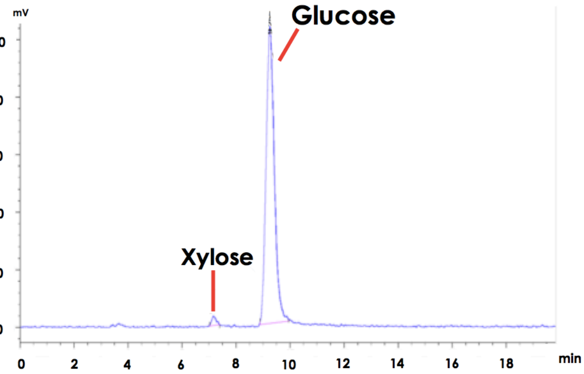

Fig 2. Chromatogram of 4 M NaOH RH-T on Zorbax carbohydrate column.

Table 4 Sugar content of 4 M NaOH RH-T.

Sugar content	Retention time (min)	Content (mg/1mL)
Xylose	7.156	0.103^{b}
Glucose	9.255	0.5917^{a}

a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.01$).

3.4 Effect of extraction process (4 M NaOH) on probiotics growth

The 4 M NaOH RH-T was used as a carbon source for the cultivation of two probiotic strains. It was found that the 4 M NaOH RH-T stimulated the growth of *L. casei* and *Bifidobacterium longum* by increasing the number from 3.85×10^8 CFU/mL to 4.30×10^8 CFU/mL and 3.39×10^8 CFU/mL to 3.90×10^8 CFU/mL within 48 h, respectively. Although 4 M NaOH RH-T stimulated the growth of two probiotic strains, it had a lower effect than positive control, oligosaccharides (FOS and XOS) and inulin (Table 5) even the pH value of 4 M NaOH RH-T decreased to 4.45. Inulin showed the greatest significant difference ($p \leq 0.05$) as inulin (ST-gel Orafti®) has 92% inulin content with a degree of polymerization ≥ 10 (Beneo, 2013). According to Aracia et al. (2011), mixing of short- and long-chain inulin improves probiotic growth.

Table 5 Microbial count of *Lactobacillus casei* and *Bifidobacterium longum*

Carbon Sources	pH before incubated		pH after incubated		Microbial counts ($x10^{8}$ CFU/mL)	
	L. casei [x]	*Bifidobacterium longum*y	*L. casei* [x]	*Bifidobacterium longum* [y]	*L. casei* [x]	*Bifidobacterium longum* [y]
Positive Control [u]	5.98 ± 0.01^{d}	5.98 ± 0.01^{d}	3.56 ± 0.01^{d}	4.62 ± 0.01^{d}	9.05^{d}	7.50^{b}
Negative Control [v]	6.21 ± 0.02^{a}	6.21 ± 0.02^{a}	5.53 ± 0.01^{a}	5.30 ± 0.01^{a}	0.24^{f}	0.20^{e}
FOS	6.16 ± 0.01^{b}	6.16 ± 0.01^{b}	3.86 ± 0.01^{c}	4.96 ± 0.01^{c}	36.00^{b}	5.20^{c}
XOS	5.63 ± 0.01^{c}	5.63 ± 0.01^{c}	5.04 ± 0.01^{b}	4.92 ± 0.01^{c}	15.00^{c}	8.90^{a}
Inulin Orafti®	6.20 ± 0.03^{a}	6.20 ± 0.03^{a}	3.82 ± 0.01^{c}	5.06 ± 0.01^{b}	51.00^{a}	8.50^{a}
RH-T (4 M NaOH)	6.22 ± 0.02^{a}	6.22 ± 0.02^{a}	4.45 ± 0.02^{b}	5.32 ± 0.02^{a}	4.30^{e}	3.90^{d}

[u] Positive Control = Glucose
[v] Negative Control = No carbon source
[x] Microbial Starter = $3.85x10^{8}$ CFU/mL
[y] Microbial Starter = 3.39 $x10^{8}$ CFU/mL
a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p\leq 0.01$).

4. CONCLUSION

Results showed that extraction by 4 M NaOH was the most efficient method for extracting the dietary fiber which gave the best physicochemical and functional properties than the control. Glucose and xylose were the main sugars found in dietary fibers extracted with 4 M NaOH and the 4 M NaOH RH-T stimulated the growth of *L. casei* and *Bifidobacterium longum*

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