Original Article

Evaluation of Dietary Fiber and Phenolic Compounds in Rice (*Oryza sativa* Linn.) and the Extracts from Rice Bran, Benjakul and Wild Betel Leaf Bush (*Piper sarmentosum* Roxb.)

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Background: The previous studies found that metabolic dysfunction in rats induced by high-fat feeding apparently subsides if they were co-fed with either the water extract from rice bran or herbs, Benjakul (BWE) or Wild betel leaf bush (WWE). The extracts contain a mixture of substances but carbohydrate was found as a majority. Therefore, the tentative active carbohydrates, dietary fiber and phenolic compounds, were evaluated.

Materials and Methods: Dietary fiber was analyzed by AOAC Method 2009.1 and 2011.25 using the reagent kits. Phenolic compounds were determined by the HPLC.

Results: The results showed that the possible active substances in RB, BWE and WWE were 2 kinds of soluble dietary fiber, the non-precipitated and the precipitated fiber in 78% ethanol, since the combination of these 2 kinds of soluble dietary fiber were approximately equal among the specimens (60.93±10.70 plus 55.56±5.33 mg/g rice; 72.72±1.36 plus 16.33±1.20 mg/g BWE; 104.38±0.54 plus 1.77±0.08 mg/g WWE). The content of phenolic compound, *p*-coumaric acid, tended to be equal among the specimens as well (38.02±2.36 µg/g rice bran enzymatic extract; 38.92±2.49 µg/g BWE; 59.019±5.42 µg/g WWE).

Conclusion: The molecular weight of the soluble dietary fibers found in RB, BWE and WWE are similar to oligosaccharides those known to be the prebiotics. The similar content of soluble dietary fiber concomitant with the similar ability to ameliorate the metabolic syndrome in the animal studies. The other possible activity to ameliorate the signs of metabolic syndrome found in the specimens is the antioxidant effect as due to *p*-coumaric acid in the extracts.

Keywords: Rice bran, Benjakul, Wild betel leaf bush, Dietary fiber, Antioxidant

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Incidence and the earlier age of onset of type 2 diabetes was found among the abdominal obese Thai[1,2]. Blood sugar reduction was found in diabetic patients whose diet containing 40 g rice bran per day for 7 days[3]. Metabolic syndrome and death rate of heart attack was lower in individuals who consumed whole grain diet than those who did not[4]. The trial earlier showed that rice bran enzymatic extract was able to reduce glycosylated hemoglobin and fasting blood glucose in type 2 diabetic patients[5].

The study in normal male Sprague-Dawley rats those were fed a high-fat diet (about 65% calories derived from fat) as well as the enzymatic extract from rice bran were able to maintain their weights and the amount of abdominal fat mass to near normal. Their abnormalities of organs and their function were subsided as well[6-11]. Similar results were found in these rats when they were orally treated with the water extract from either a mixed Thai herbs, Benjakul[12], or a single herb, Wild betel leaf bush[12,13]. It was generally believed that the benefit of eating brown rice or any vegetables on obesity including those abdominal ones was mostly due to the roughage dietary fiber contents in those foods. However, the studies on the water extracts mentioned above were shown the impressive effects on those abdominal obese rats as well. It is, therefore, interesting to identify the soluble dietary fiber in rice and those extracts. Since the proximate analysis of these water extracts showed that the majority substance was carbohydrates, the amount and types of sugars was identified in rice bran in comparison to the whole grain as brown rice (unpolished) and white rice (polished) of the same variety as well. The other possible benefit of plant materials to prevent liver damage from being fed with a high-fat diet is via the anti-oxidation. So, the content of some phenolic compounds were assessed in rice and herbal extracts as well.

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Materials and Methods

Plant collection and preparation of extracts

All plant materials were collected from farms with Organic Agriculture Certification, Ministry of Agriculture and Cooperative, Bangkok, Thailand. Plant authenticity was verified by the Royal Forest Department, Ministry of National Resources and Environment, Bangkok, Thailand. The rice variety *Oryza sativa* Linn. Khao Dawk Mali 105 and its products was purchased from an organic farm in Srisaket Province: BR, WR, and rice bran. Rice bran enzymatic extract (RBEE) was prepared as described by Kandee et al[6]. Enzymes in fresh bran were inactivated in a hot-air oven at 130 to 140°C for 3 min; then, 120 g of bran was stirred in 480 mL of 70°C distilled water for 1 h. The suspension was centrifuged at 11,485 x g at 25°C for 10 min. The supernatant was transferred to a beaker and 7 mL of mixed enzymes, then 10 mg of α-amylase dissolved in 6.5 mL distilled water plus 5 mL of amyloglucosidase solution (Sigma-Aldrich) was added. The mixture was stirred at 37°C for 30 min; 3.0 mL of Tris Base 0.75 M was added at 60°C to terminate the reaction. Then, it was centrifuged at 11,485 x g at 25°C for 10 min before the supernatant was freeze-dried (Freezone 18, Labconco Ltd., USA). Approximately 12.2 g % of dried powder was obtained. Rice bran ethanol extract (RBEtE) was created by mixing rice bran with 95% ethanol in a ratio of 1 g: 10 mL. After leaving it at room temperature for 8 h, the mixture was filtered. The filtrate was then evaporated, and the dry powder of the extract was obtained.

Maha-pigud-soros-benjakul, Benjakul, consists of 36 variable weighing units, and 5 unique formulas may be given depending on the origin of the illness: earth/solids (Patavee), water/liquids (Arpo), wind/gas (Vayo), fire/heat (Techo) and vacuum/ether (Arkad). We have chosen Arpo as it is given for diabetes (Table 1).

Plant parts and whole plants were separately washed, chopped, dried in the hot air oven at 55°C and ground. Benjakul was prepared in accordance with the formula weights in Table 1. Twice the volume of distilled water was added. Then, it was boiled at 100°C (decoction) until half the water volume was left. This filtrate was freeze-dried at -20°C; the pellet, or BWE, was weighed. The same treatment was done with the whole plant of Wild betel leaf bush to obtain WWE. Approximately 6.7 and 3.3 g% of dried extract was the yield from Benjakul and Wild betel leaf bush, respectively.

Determination of dietary fiber

Dietary fiber was assessed using the Integrated Total Dietary Fiber Assay Kit (Megazyme International, Ireland, 2015)[14,15]. Briefly, high molecular weight of carbohydrate and protein, if they have any, were digested with enzymes (pancreatic α-amylase, amyloglycosidase and protease). After digestion, it was divided into 2 parts, one for the analysis according to AOAC Method 2009.1 and the other for AOAC Method 2011.25.

The AOAC Method 2009.1 starts with pre-heating the sample to 60°C and precipitating it by adding 95% aqueous ethanol, leaving it at room temperature for 60 min to achieve suspension: the approximate final concentration of ethanol was 78%. Then, it was filtered through a pre-weighed crucible containing Celite®. The protein and ash weights were assessed and subtracted from the precipitate to calculate high molecular weight dietary fiber (HMWDF).

The second liquid portion was analyzed according to AOAC Method 2011.25. The digested specimen was filtered through a pre-weighed crucible containing Celite®. The precipitate obtained after washing the crucible with 60°C distilled water is indigestible dietary fiber (IDF). The remaining filtrate was warmed to 60°C and 95% ethanol was added (the approximate final concentration of ethanol was 78%). After leaving the mixture at room temperature for an hour, the precipitate of soluble dietary fiber (SDFP) was gained.

Filtrate from both methods was assessed for soluble dietary fiber (SDFS), which is soluble in both water and alcohol. The SDFS solution was evaporated to dryness under vacuum at 60°C, re-dissolved in 150 mM HCl to give a pH of 4.5, then digested by amyloglucosidase and deionized through a column (Econo-Pack® Disposable Economy Columns, Bio-Rad, USA) packed with mixed-bed ion exchange resins, Amberlite® FPA 53 (OH^-) and Ambersep® 200 (H^+) (Rohm and Haas, France SAS) using a pump (Gilson Minipuls® Evolution, USA). The eluate was evaporated under vacuum at 60°C to dryness. The pellet was dissolved in 2 mL of deionized water.

Determination of SDFS and carbohydrate molecules

The filtrate was quantitate for SDFS and carbohydrate molecules using high performance liquid chromatography (HPLC) equipped with refractive index detector, 50 mL injection loop, a pump (Agilent 1260, CA, USA) and the 6.5x300 mm Waters Sugar-Pak® column (Waters

Table 1. The dried weight of Arpo formula plant parts

Common name	Scientific name	Part of plant	Weighing unit
Long pepper	*Piper retrofractum* Vahl	Fruit	2
Wild betel leaf bush	*Piper sarmentosum* Roxb	Root	16
Pepperwood (sic)	*Piper interruptum* Opiz	Stem	8
Indian leadwort	*Plumbago indica* Linn	Root	6
Ginger	*Zingiber mekongense* Gagnep	Rhizome	4

Corporation, MA 01757, USA). Distilled water containing 50 mg/L Na_2Ca-EDTA was used as a solvent with a flow rate of 0.5 mL/min at 90°C. The peak of LMWSDF, maltose + fructosyl-trisaccharide, glucose and sorbitol were identified as described by McCleary et al[15]. The area under the curve was calculated for each substance in each fraction by comparing that with an internal standard, sorbitol.

Determination of phenolic acids

Phenolic acids in the extracts were determined by HPLC equipped with a UV detector, ConstaMetric 4100 Pump, the SpectroMonitor 4100 Detector at 280 nm and an auto sampler AS3500 (Thermo Separation Products-TSP, Riviera Beach, CA, USA). The C18 column used was 4.6x250 mm (5 µm) with guard (Phenomenex, USA). The process was performed at room temperature using 1% acetic acid and acetonitrile as the gradient mobile phase[16]. Ten µL of each sample were analyzed in triplicate. The area under the curve was calculated using ChromeQuest software (Thermo Separation Products TSP, Riviera Beach, CA, USA). The standard phenolic compounds were obtained from (Sigma-Aldrich, Germany).

Determination of catechins

Catechins in the extracts were determined by HPLC as phenolic acids[17]. Then the mobile phase consisted of formic acid solution (pH 2.5, A) and methanol (B). Elution was performed with a linear gradient by decreasing A from 80% to 40% within a period of 15 min, isocratic at 90% solvent B from 15.1 to 22 min, and re-equilibration period of 8 min with 80% solvent A were used between individual runs.

Results

Sorbitol was co-analyzed as an internal standard; and 3 specimens were used for each sample group. The *p*-value was obtained from the analysis of variance.

Fractions identified, except for glycerol, differed significantly between samples. HMWDF is a combination of water-insoluble dietary fiber (IDF) and that is also insoluble in 78% ethanol (SDFP). LMWSDF, the larger molecules than maltose and tri-saccharide was identified in the filtrate after HMWDF precipitation. Such filtrates did not only contain only LMWSDF but also smaller tri-saccharides e.g. fructosyl-tri-saccharide (F3), the hydrolysis product of inulin, sugars, and glycerol. Since the peak of maltose is quite high and the area under the curve is quite broad, the real peak of F3 cannot be identified; thus, the area at the peak of maltose represents levels of both maltose and F3.

RB had the highest amount of HMWDF, which was mostly IDF, while WWE had the second highest level, mostly being SDFP. RB also had the highest level of LMWSDF.

Phenol amounts and types varied according to plant species, but *p*-coumaric acid and some catechins were commonly found throughout the samples. Most of the phenolic compounds from Wild betel leaf were extracted by water decoction with WWE containing the most *p*-coumaric acid, ferulic acid, ECG and CG. Rice bran using ethanol extraction (RBEtE) demonstrated the highest levels of gallic acid and EGC.

Discussion and Conclusion

The verification of dietary fiber (Table 2) and phenolic compounds (Table 3) content in rice, RBEE, RBEtE, BWE and WWE, found that the amount of soluble dietary fiber, SDFP plus LMWSDF, and *p*-coumaric acid were more or less equally found in RB (presumably, it represented the amount in RBEE), BWE and WWE. A member of LMWSDF were small molecules of carbohydrate such as oligosaccharides. Since these substances in RBEE were poorly digested or absorbed in the human gut[18], therefore, they might be able to act as prebiotics. Moreover, substances defined as SDFP (higher molecular weight than substances in LMWDF) might be able to inhibit fat and sugar absorption in the gut as well. The highest content of HMWDF in RB could also reduce constipation, clean the gut and promote gut movement. It is interesting that the number of HMWDF and SDFP was high in both water extracts, WWE and BWE. This brought to the assumption that RBEE and RBEtE should have the same

Table 2. Weights (mean ± SD) of dietary fiber and other carbohydrate compounds in rice bran (RB), white rice (WR), brown rice (BR), Benjakul water extract (BWE) and water extract of Wild betel leaf bush (WWE)

Mg/g	RB	BR	WR	BWE	WWE	*p*-value
HMWDF	255.57±7.92	36.06±0.35	7.42±0.43	99.41±0.74	109.91±0.78	<0.005
IDF	192.80±0.69	29.58±0.94	4.93±0.37	26.69±0.62	5.53±0.30	<0.005
SDFP	60.93±10.70	6.48±0.64	2.48±0.13	72.72±1.36	104.38±0.54	<0.005
LMWSDF	55.56±5.33	30.32±2.62	18.55±0.71	16.33±1.20	1.77±0.08	<0.01
Maltose+F3	283.09±4.91	535.56±4.04	591.02±11.48	192.87±6.03	32.38±0.37	<0.005
Glucose	14.28±0.26	18.59±4.22	24.82±2.84	62.96±0.38	3.54±0.02	<0.025
Glycerol	168.87±3.03	174.62±4.39	178.22±2.68	182.44±8.18	186.65±0.70	ns
Sorbitol	97.03±0.02	97.00±0.04	97.02±0.03	97.01±0.01	97.01±0.01	ns

F3 = fructosyl-trisaccharide, ns = non-significant

Table 3. Weights (mean ± SD) of phenolic acids and catechins in rice bran enzymatic extract (RBEE), rice bran ethanol extract (RBEtE), BWE and WWE

mg/g	RBEE	RBEtE	BWE	WWE	*p*-value
p-coumaric	38.02±2.36	23.63±3.26	38.92±2.49	59.019±5.42	ns
Ferulic	ND	34.24±1.27	18.07±2.20	1,181.83±6.30	<0.01
Sinapic	ND	13.63±3.41	18.09±0.58	12.23±0.04	<0.001
Gallic acid	33.63±0.41	398.97±9.14	ND	76.32±0.31	<0.001
EGC	ND	576.16±4.63	ND	ND	<0.001
EGCG	ND	3.40±1.18	56.57±4.11	ND	<0.001
ECG	137.66±4.55	282.38±31.48	ND	751.28±11.48	<0.005
CG	ND	ND	160.58±13.12	586.08±7.68	<0.001

Catechins include gallic acid
EGC = Epigallo catechin, EGCG = Epigallo catechin gallate, ECG = Epicatechin gallate and CG, CG = Catechin gallate, ND = not-detected. The number of specimen is 3 for each sample group. The *p*-value was obtained from the unpaired t-test and "ns" is non-significant.

amount of SDFP as that found in RB. Therefore, apart from essential substances from LMWSDF fraction, such substances from SDFP should exist in RBEE as well. Altogether, substances in LMWSDF and SDFP were supposed to ameliorate the symptom of metabolic syndrome in rats fed a high-fat diet. The certain amount of *p*-coumaric acid in all extracts studied would emphasized their function as antioxidant in gut and possibly protect gut function.

Finally, it should be kept in mind that not only carbohydrate substances in RB, RBEE, BWE and WWE that could ameliorate the metabolic abnormalities in rats fed a high-fat diet, but there were amino acids and some minerals those could play the role as well.

What is already known on this topic?

Metabolic dysfunction in rats induced by high-fat feeding apparently subsides if they were co-fed with either the water extract from rice bran, Benjakul or Wild betel leaf bush.

What this study adds?

Soluble dietary fibers found in RB, BWE and WWE are similar to oligosaccharides. The other possible activity is the antioxidant effect as due to p-coumaric acid in the extracts.

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Potential conflicts of interest

The bauthors declare no conflicts of interest.

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การประเมินปริมาณเส้นใยอาหารและสารประกอบฟีโนลิกในข้าว (*Oryza sativa* Linn.) และในสารสกัดจากรำข้าวเบญจกูลและช้าพลู (*Piper sarmentosum* Roxb.)

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ภูมิหลัง: จากงานวิจัยก่อนหน้านี้ที่มีการทดลองในหนูพบว่าหนูทดลองที่ได้รับอาหารไขมันสูงจะเกิดกลุ่มอาการผิดปกติทางระบบเมแทบอลิซึม แต่เมื่อหนูเหล่านี้ได้รับสารสกัดจากรำข้าว เบญจกูล หรือช้าพลู พร้อมกันอาการผิดปกติที่พบจะลดลง จากการศึกษาพบว่าสารสกัดทั้งสามชนิดประกอบด้วยสารคาร์โบไฮเดรตเป็นหลัก จึงคาดว่าสาระสำคัญในสารสกัดเหล่านี้น่าจะเป็นสารจำพวกเส้นใยอาหารและสารประกอบฟีโนลิก

วัสดุและวิธีการ: ทำการวิเคราะห์ปริมาณสารประกอบเส้นใยอาหารในรำข้าว ข้าวสาร ข้าวกล้อง สารสกัดจากรำข้าว สารสกัดจากเบญจกูล (BWE) และสารสกัดจากช้าพลูทั้งต้น (WWE) และทำการวิเขราะห์ปริมาณสารประกอบฟีนอลิก.

ผลการศึกษา: พบว่าเส้นใยอาหารที่ละลายได้ในน้ำซึ่งนับรวมเส้นใยอาหารที่ละลายในน้ำได้แต่ตกตะกอนในแอลกอฮอล์เข้าด้วยกัน มีค่าเกือบเท่ากันในตัวอย่างทั้งสาม คือ 60.93±10.70 บวก 55.56±5.33 มก./ก. รำข้าว; 72.72±1.36 บวก 16.33±1.20 มก./ก. BWE; 104.38±0.54 บวก 1.77±0.08 มก./ก. WWE

สรุป: จากผลการศึกษาคาดว่าหนึ่งในสารประกอบเส้นใยอาหารที่พบในตัวอย่างทั้งสาม ได้แก่ สารโอลิโกแซ็กคาไรด์ ซึ่งเป็นสารที่มีคุณสมบัติเป็นอาหารให้แก่จุลชีพประจำถิ่นของลำไส้ส่งเสริมภูมิคุ้มกันแก่ลำไส้และควบคุมการดูดซึมของเสีย รวมทั้งยังมีสารประกอบฟีโนลิกชนิด p-coumaric acid ซึ่งมีฤทธิ์ต้านอนุมูลอิสระในสารสกัดจากรำข้าว เบญจกูลและช้าพลู ในปริมาณเกือบเท่ากันอีกด้วย จึงเชื่อว่าเส้นใยอาหารที่ละลายในน้ำ และ p-coumaric acid คือ สารสำคัญที่สามารถป้องกันการเกิดกลุ่มอาการทางเมแทบอลิซึม