Production of Fiber Hydrolysate from Bamboo Shoot with Antioxidative Properties by Enzymatic Hydrolysis

Supatra Karnjanapratum[1], Pensiri Kaewthong[1], Sirima Takeungwongtrakul[2], Thanasak Sae-leaw[3], Jeong Hwa Hong[4] and Sitthipong Nalinanon[1*]

[1]Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
[2]Department of Agricultural Education, Faculty of Industrial Education and Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
[3]Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[4]Department of Smart Foods and Drugs, School of Food and Life Science, Inje University, Gimhae, South Korea

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Abstract

Bamboo shoots possess a rich source of dietary fiber for Asian countries as well as Thailand, which give various beneficial physiological effects for human beings. Dietary fibers in soluble form could provide better texture and would be easier to apply in food products. This study aimed to prepare fiber hydrolysate with high solubility and antioxidant activity from bamboo (*Bambusa vulgaris*) shoot. The fiber hydrolysate from bamboo shoot (FHBS) was prepared by stepwise enzymatic hydrolysis including amylase (1%, w/w), cellulase (1, 2, 3%, w/w) and papain (1%, w/w). The released fiber yield of FHBS increased with increasing cellulose levels in dose dependent manner ($P \leq 0.05$). It was found that the process with 1% (w/w) amylase for 1 h and 3% (w/w) cellulase for 3 h followed by 1% (w/w) papain for 1 h at 50 ºC, rendered the highest released fiber yield (92.10±1.10%). The resultant FHBS contained 5.76±0.21 % of total dietary fiber with total sugar and reducing sugar contents of 1431.22±46.01 and 918.91±10.57 mg/g solid, respectively. The FHBS exhibited antioxidant activities including ABTS radical scavenging activities (ABTS), DPPH radical scavenging activities (DPPH), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC). Therefore, FHBS with antioxidant activities could be effectively prepared by using enzymatic hydrolysis and suitable to apply in the fiber fortified products.

Keywords: bamboo shoot, fiber hydrolysate, antioxidative properties, enzymatic hydrolysis
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*Corresponding author: Tel.: +66 2329 8000 ext 7253 Fax: +66 2329 8527
E-mail: sitthipong.na@kmitl.ac.th

1. Introduction

Dietary fiber is part of daily food intake, normally from part of plant material that is resistant for digestion by human digestive system [1]. Even though it is carbohydrate including cellulose, noncellulosic polysaccharides and non-carbohydrate component lignin, fiber cannot be broken down into sugar molecules like other typical carbohydrates due to its structure [2]. High-fiber diets are growing in popularity as compared to low-fat or low-calorie diets and integrating healthy nutrition within regular daily routines via small changes to consumer's lifestyles are needed. Fiber also comes in two forms; soluble and insoluble [3]. Insoluble form cannot be digested or absorbed by human bodies and is insoluble in water, which can be used as bulk agent for products to prevent constipation. Soluble fibers dissolve in water and can be used by human bowel bacteria as their food source. The soluble form has outstanding health promotion functions [3, 4]. Moreover, soluble dietary fiber could properly be fortified as functional ingredient in food products, especially in beverages, which are a convenient and efficient delivery vehicle for many essential nutrients such as antioxidant plant extracts and protein/peptides as well as soluble dietary fiber [1, 5-7].

Non-starch polysaccharides in plant, particularly fiber, with antioxidant properties have been exploited as potential novel antioxidants. Several rice bran fiber fractions offer protection against the superoxide radical, hydroxyl free radical, lipid peroxidation and exhibit good potential for reducing power and chelating ferrous ions [8]. Besides nutrients, bamboo shoots also contain lethal concentration of oxalate content and the anti-nutrient (cyanogen) that need to be removed before human consumption, which could effectively be removed by boiling in water [9, 10]. Bamboo shoot dietary fiber is an inexpensive alternative fiber apart from wheat, oat, corn, soybean and apples, especially for Asian people. There are many studies reported on its extensive biological activities such as antibacteria, antitumor, anticancer, immune regulation and so on [11]. However, the dietary fiber in bamboo shoots is mostly insoluble, which limits its applications in food products. There are several techniques used to modify and increase soluble dietary fiber of bamboo shoot and the enzymatic hydrolysis was found as an effective method to improve physicochemical properties and the bioactivity of resulting fiber [3, 12, 13]. Xiao-bing *et al.* [12] evaluated the effects of different preparation methods, including water-washing, acid-base treatment, fermentation and enzyme treatment, on the quality of dietary fiber from bamboo shoot. They found that the highest contents of soluble, insoluble and total dietary fibers were obtained from enzyme treatment with alpha-amylase and papain. Song *et al.* [13] reported that the incorporation of extrusion with enzymatic hydrolysis by cellulase could increase the contents of soluble dietary fiber (22.17 g/100 g dry solids) of amylase and papain pretreated bamboo shoots, and the resulting fiber can be useful as a fiber-rich ingredient in functional foods. Alpha-amylase could rapidly increase starch hydrolysis [14]. Papain is a cheap cysteine protease obtained from the latex of papaya, which can cleave protein and peptide in bamboo shoot matrix. These two enzymes have been used for pretreatment of the bamboo shoot and provided the matrix which is prone to be modified by extrusion, chemical or enzymatic methods [13, 15] In addition, cellulase can hydrolyze cellulose and hemicellulose components, which most likely leads to increase exposure of functional groups and influences the bioactivity of dietary fiber [16]. Therefore, the aim of this study was to prepare the soluble dietary fiber from bamboo shoot by stepwise hydrolysis with several enzymes. The antioxidative activities of resultant fiber hydrolysate were then evaluated.

2. Materials and Methods

2.1 Chemicals

Amylase (enzyme activity: 10,000 U/g) and papain (enzyme activity: 100,000 U/g), were obtained from Shaanxi Orient Industrial Co., Ltd. (Shaanxi, China). Cellulase (enzyme activity: 20,000 U/g) was purchased from the Beijing Aoboxing Biotechnology Co., Ltd. (Beijing, China). The other reagents were of analytical grade.

2.2 Bamboo shoot preparation

The fresh bamboo shoots were purchased from local market in Ladkrabang, Bangkok, Thailand and transported to Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang. The bamboo shoots were peeled and washed with running water before cut into pieces with a diameter of 5 cm. Bamboo shoots were then boiled in hot water (95 ºC) for 15 min prior to storage in polyethylene bag with vacuum seal. The prepared bamboo shoot was stored at -20 ºC until used for analysis within 6 months.

2.3 Pretreatment

Two hundred grams of prepared bamboo shoot were mixed with 400 ml of distilled water and homogenized into slurry. The slurry was then boiled for 15 min and filtered through two-layer of cheesecloth. This process was done twice. The moisture content of pretreated bamboo shoot was analyzed and calculated to be 88.94 ± 2.41%.

2.4 Preparation of fiber hydrolysate by enzymatic hydrolysis

The pretreated bamboo shoot (150 g) was homogenized into slurry with 450 ml water (pH 5.1-5.5). The slurry was preheated at 50°C in water bath. Then, the bamboo shoot slurry was hydrolyzed at 50°C by stepwise enzyme treatment including α-amylase (1% w/w) for 1 h, cellulase (1, 2 and 3% w/w) for 3 h and papain 1% (w/w) for 1 h, respectively. The resultant mixture was heated at 95°C for 15 min for enzyme inactivation prior to filter to remove insoluble debris. The filtered mixture was referred to as "fiber hydrolysate" and collected for analysis.

2.5 Released fiber hydrolysate yield

The released fiber hydrolysate yield was calculated based on initial weight (wet weight) of the starting material using the following equation:

$$\text{Released fiber hydrolysate yield (\%)} = \frac{\text{Total solid of fiber hydrolysate (g)}}{\text{Total weight of bamboo shoot (g)}} \text{x} 100$$

2.6 Proximate analysis

The methods for determining the chemical composition of bamboo shoot and its hydrolysate from selected condition, including moisture, protein, fat and ash contents are outlined in the Official Methods of Analysis [17]. Total carbohydrate was calculated by subtraction sum of protein, fat moisture and ash from total weight of sample [18].

2.7 Total sugar and reducing sugar contents

Total sugar content was determined by the phenol-H_2SO_4 method [19] by measuring the absorbance at 490 nm, using sucrose as a standard. The reducing sugar contents was evaluated as per DNS (3,5-dinitrosalicylate) method [20] using glucose as a standard.

2.8 Fiber composition analysis

The fiber compositions of the fiber hydrolysate from bamboo shoot (FHBS) were evaluated from the hydrolysate without filtration. The insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and total dietary fiber (TDF) were determined using the Megazyme TDF Test Kit (K-TDFR, Megazyme International Ireland, Bray Business Park, Bray, Co., Wicklow, Ireland) exactly according to enzymatic gravimetric method with MES-TRIS buffer, based on the AOAC991.43 [21].

2.9 Antioxidative activities

Antioxidative activities of the sample were determined for ABTS radical scavenging activity (ABTS) [22], DPPH radical scavenging activity (DPPH) [22] and ferric reducing antioxidant power (FRAP) [23]. The oxygen radical absorbance capacity (ORAC) was also determined as per method of Kittiphattanabawon *et al.* [24]. Trolox (50 mg/ml) was plotted between relative fluorescence intensity (%) and time (min). All activities were expressed as mmol Trolox equivalent (TE)/g sample.

2.10 Statistical analysis

All experiments were done in triplicate using three different lots of bamboo shoot. Pairwise T-Tests were performed for evaluating the differences in chemical composition of boiled bamboo shoot and FHBS. Significant differences among means within each experiment were evaluated by Duncan's multiple range test at a significance level of $\alpha = 0.05$ [25]. Statistical analysis was performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 Released fiber hydrolysate yield

Fiber hydrolysates from bamboo shoot (FHBS) obtained by stepwise enzymatic hydrolysis with variation of cellulase concentrations, are shown in Figure 1 and those released fiber hydrolysate yield counterparts are presented in Figure 2. The result showed that greater amount of bamboo shoot hydrolysis occurred with higher amount of enzyme used, as monitored by the decreasing of sample mass (Figure 1). The remained sample masses were 17.53±1.35%, 10.89±1.13% and 5.04±0.67% after enzymatic hydrolysis using 1%, 2% and 3% of cellulase, respectively, which were significantly lower than that from control (0% cellulase; 94.36±5.53%). From control, the lowest yield of soluble fraction was obtained ($P \leq 0.05$), compared to enzymatic treatments (Figure 2). The yield of FHBS increased with increasing cellulase levels in dose dependent manner ($P \leq 0.05$). The highest cellulase concentration used (3%) resulted in the highest yield (92.10±1.10%). This result indicated that the cellulose in bamboo shoot matrix could be cleaved by cellulase, in which the short chain fractions

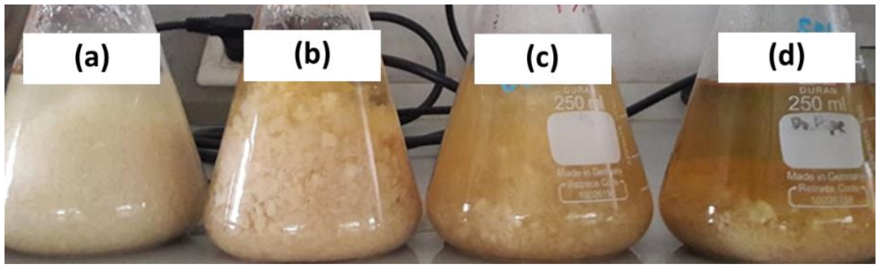

Figure 1. Fiber hydrolysate of bamboo shoot (FHBS) prepared by stepwise enzymatic hydrolysis with different concentrations of cellulase. (a) 1% (w/w) α-amylase and 1% (w/w) papain, (b) 1% (w/w) α-amylase, 1% (w/w) cellulase and 1% (w/w) papain, (c) 1% (w/w) α-amylase, 2% (w/w) cellulase and 1% (w/w) papain, (d) 1% (w/w) α-amylase, cellulase (3% (w/w) of shoot mass) and 1% (w/w) papain

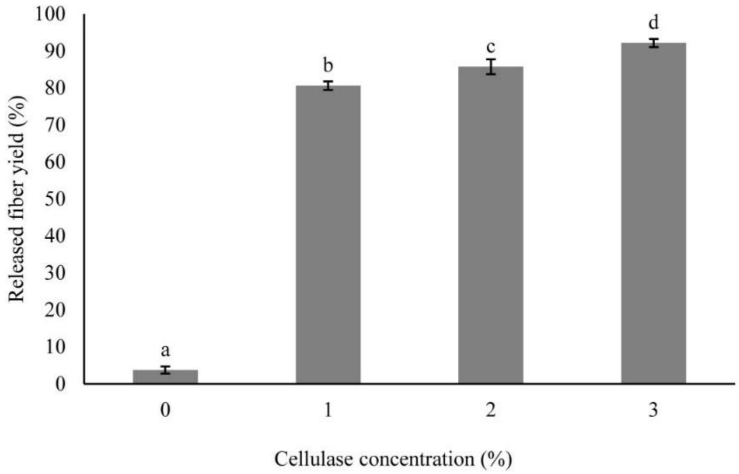

Figure 2. Released fiber hydrolysate yield (%) from bamboo shoot by stepwise enzymatic hydrolysis with different concentrations of cellulase. Different small letters on the bars indicate significant differences (P≤0.05).

with greater solubility could be obtained. Glucose and cellobiose are two major products from enzymatic hydrolysis of cellulose by cellulase [26]. Two major steps (including adsorption of enzymes onto surfaces of cellulose and breakage of β-1,4-glucosidic bond between glucose) are involved in enzymatic hydrolysis of cellulose [27]. The result was in accordance with those reported by Shafiei *et al.* [28] who prepared palm date fibers by using enzymatic hydrolysis. Cellulase with higher concentration (50 FPU/g activity) was able to hydrolyse glucan in the fibers with higher degree (67.1%), compared with those from 20 FPU/g activity (37.5%). Therefore, the stepwise enzymatic hydrolysis using 1% (w/w) α-amylase, 3% (w/w) cellulase and 1% (w/w) papain was the suitable enzyme treatment condition and was also selected to use for the production of FHBS for further analysis.

3.2 Chemical compositions

Chemical compositions of boiled bamboo shoot and the selected fiber hydrolysate (FHBS) obtained from stepwise enzymatic hydrolysis using 1% α-amylase, 3% cellulase and 1% papain, are shown in Table 1. Both samples (boiled bamboo shoot and FHBS) had carbohydrate as a major composition, followed by protein and ash contents, respectively. No fat content was detected from bamboo shoot and FHBS. These results were in accordance with chemical compositions of bamboo shoot reported by Nongdam and Tikendra [29] and Wang *et al*. [15]. Carbohydrate and protein are main composition found from boiled bamboo shoot with various amount depended on types and cooking processes, in which fat content could not be detected from all boiled shoot tested [29]. It was noted that FHBS had higher protein and ash contents, compared with those of substrate used (boiled bamboo shoot) ($P \leq 0.05$). This might indicate the residue of protein and ash content from enzyme added during stepwise enzymatic hydrolysis.

Table 1. Chemical compositions of boiled bamboo (*Bambusa vulgaris*) shoot and the selected fiber hydrolysate from bamboo shoot (FHBS).

Chemical compositions	Boiled bamboo shoot	FHBS	t-test
Ash (% dry basis)	1.23 ± 0.01	2.35 ± 0.12	*
Protein (% dry basis)	5.23 ± 0.11	6.19 ± 0.12	*
Fat (% dry basis)	ND	ND	-
Carbohydrate (% dry basis)	93.5 ± 0.10	91.5 ± 0.14	*

Values are expressed as means ± standard deviation. *Significant differences between means of boiled bamboo shoots and FHBS ($P \leq 0.05$). ND = not detected.

3.3 Sugar and fiber composition

Sugar and fiber compositions of FHBS prepared by selected condition are shown in Table 2. The FHBS contained 892 ±14.2 mg/g of total sugar. In addition, this hydrolysate consisted of high content of reducing sugar, relating to the high degree of degradation of starch and cellulose by amylase and cellulase, respectively, in which the glucose and oligomers could be released. Some enzymes produced naturally by microorganisms could reduce the molecular weight and improve the solubility of dietary fiber [30].

After stepwise hydrolysis, the FHBS without filtration had 5.76±0.21% of total dietary fiber, including 1.30±0.09% and 4.46±0.20% of soluble and insoluble dietary fiber, respectively. This result indicated that bamboo shoot fiber was almost cleaved during enzymatic hydrolysis to generate soluble fraction in the extracted fractions. The presence of monomer sugars and oligomers from complete and incomplete hydrolysis of polysaccharides could be obtained from palm date fiber hydrolysate by using cellulase and resulted the soluble fraction [28]. Enzymatic treatment hydrolyzes the insoluble fiber into soluble fraction, which could improve the prebiotic health benefits of the developed product [31]. Ramos *et al.* [32] found that a product from cocoa husks treated with the enzyme mixture Ultraflo L® resulted in soluble cocoa fiber which showed the potential application as a functional food ingredient. Therefore, FHBS might exhibit some promising biological activities.

Table 2. Sugar and fiber compositions of the selected fiber hydrolysate from bamboo shoot (FHBS)

Characteristics	**Contents***
Total sugar[£] (mg/g solid)	892 ± 14.2
Reducing sugar content[£] (mg/g solid)	735 ± 8.45
Total dietary fiber content[¥] (g/100 g solid)	5.76 ± 0.21
Soluble dietary fiber content[¥] (g/100 g solid)	1.30 ± 0.09
Insoluble dietary fiber content[¥] (g/100 g solid)	4.46 ± 0.20

*Values are expressed as means ± standard deviation ($n = 3$). [£] The data was determined from soluble fraction. [¥] The data was determined from FHBS without filtration.

3.4 Antioxidative activities

Antioxidative activities of the selected FHBS were evaluated using different assays and presented in Table 3. It was found that the enzymatic hydrolysis produced antioxidative components that could both scavenge the radicals and work as electron donator. The bioactive oligosaccharides for regulation of plant cell growth and induction of phytoalexins were reportedly produced from primary cell-wall polysaccharides [33]. Reddy and Krishnan [34] reported on the production of prebiotics and antioxidants as health food supplements from lignocellulosic materials using multi-enzymatic hydrolysis. They found that a multi-enzyme mix produced from a new strain of *Bacillus subtilis* KCXOO6 could effectively produce prebiotic functional oligosaccharides and antioxidants from wheat bran, sugarcane bagasse, rice husk and bamboo bagasse. Previous researches have demonstrated that degraded polysaccharides by enzymatic hydrolysis process exhibited superior free radical scavenging effect [35, 36]. Moreover, the biological activities of polysaccharides are closely related to their Mw distributions. Theoretically, low Mw polysaccharides are more active than high Mw polysaccharides, which is attributed to the greater surface area and better water solubility [37-39]. Tabarsa *et al.* [40] found that the lower molecular weight of water-soluble polysaccharide fraction from *Ulva intestinalis* was very pivotal for its high bioactivity. Chen *et al*. [37] showed that the degraded polysaccharide from *Sargassum fusiforme* possesses superior anti-tyrosinase activity and antioxidant activity than the original polysaccharide. The different antioxidative properties may be due to the combined effects of the different sizes of the electron cloud density and the different accessibility between free radical and low molecular polysaccharides which, in turn, depends upon the different hydrophobicities of the constituent sugars [41]. The uncontrolled production of free radicals is involved in various diseases, including cancer, atherosclerosis and degenerative aging processes. These results clearly establish the possibility that water-soluble polysaccharides fractions from enzymatic hydrolysate of bamboo shoot might effectively be employed as ingredient in health or functional food to alleviate oxidative stress.

Table 3. Antioxidative activities of the selected fiber hydrolysate from bamboo shoot (FHBS)

Antioxidative activities	Content
ABTS (mmol TE/g solid)	16.4 ± 1.55
DPPH (μmol TE/g solid)	25.4 ± 0.01
FRAP (mmol TE/g solid)	6.24 ± 0.32
ORAC (mmol TE/g solid)	3.39 ± 0.04

*Values are expressed as means ± standard deviation ($n = 3$). ABTS: ABTS radical scavenging activity; DPPH: DPPH radical scavenging activity; FRAP: Ferric reducing antioxidant power; ORAC: Oxygen radical absorbance capacity.

4. Conclusions

The production of fiber hydrolysate from bamboo shoot with antioxidative activity was simply done by using stepwise enzymatic hydrolysis. Cellulase tended to be the key enzyme used in the hydrolytic process. The information gained could be used for soluble fiber production with antioxidative activity from bamboo shoot and other fiber sources, which had the potential to apply into food products.

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