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Characterization of Bestak sweet potato (*Ipomoea batatas*) variety from Indonesian origin as prebiotic

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Abstract

The objectives of this study were to analyze the macronutrients composition, dietary fiber, and resistant starch in Bestak sweet potato variety; to identify the prebiotic components in sweet potato fiber extract (SPFE); and to evaluate the prebiotic activity of SPFE. Evaluation of prebiotic activity was based on the change in cell biomass after 24 h of growth of the probiotic strain in the presence of SPFE, inulin, fructooligosacharide, or glucose relative to the change in cell biomass of *Escherichia coli* grown under the same condition. Prebiotic activity was calculated for *L. plantarum* Mut7 and *Bifidobacterium longum* JCM 1217. The results showed that ash, starch, and total carbohydrate in SPFE were lower than in the raw sweet potato and its powder. However the crude protein in SPFE was higher than in the raw sweet potato and its powder. SPFE contained 3.50% soluble fiber, 12.17% insoluble fiber, 22.23% resistant starch, and 37.90% total dietary fiber. The increasing cell number of *L. plantarum* Mut7 was higher in the SPFE substrate (3.21 log CFU/ml), whereas *B. longum* grew better in FOS substrate (2.19 log CFU/ml). The highest prebiotic activity score was obtained for *L. plantarum* Mut7 grown on SPFE (1.62), whereas the lowest score was for *Bifidobacterium longum* grown on inulin (0.47). It can be concluded that Bestak sweet potato variety has potency as prebiotic source because it contains FOS, inulin and raffinose. The SPFE has prebiotic activity score that similar to FOS, but it was higher than inulin.

Introduction

Gibson *et al.* (2004) defined prebiotic as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health". Prebiotic components such as fructooligosaccharide (FOS), inulin, raffinose, and others are naturally found in several plants such as tubers. There are several type of tubers from Indonesian origin that have potency as prebiotic sources. Those tubers are sweet potatoes (*Ipomoea batatas*), arrowroot (*Marantha arundinaceae*), canna (*Canna edulis*), etc. The tubers contains high amount of oligosaccharides and polysaccharides. However, there are several criteria that must be met so that dietary carbohydrates could be classified as a prebiotic. According to Gibson *et al.* (2004), the criteria are: resists gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption, is fermented by the intestinal microflora and stimulates selectively the growth and / or activity of intestinal bacteria associated with health and wellbeing.

The prebiotic activity could be determined with *in vitro* and *in vivo* method. Huebner *et al.* (2007) developed a simple method to determine the prebiotic activity *in vitro*. This method is more simple than the previous methods (Olano-Martin *et al.*, 2002, Palframan *et al.*, 2003, Vulevic *et al.*, 2004) because it does not require fecal samples. The prebiotic activity score showed the ability of a given substrate to support the growth of an organism relative to other organisms and relative to growth on a non-prebiotic substrate such as glucose. *In vivo* study of prebiotic activity usually could be done with animal and human subject. In animal subjects, the prebiotic components were orally administered and the animals were then anaesthetized and sacrificed for collection of fecal samples and gastrointestinal contents. Studies using

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human subjects were done with two approaches. The first approach is measuring the concentration of gases, especially H_2, in subjects given oral dose of the prebiotic. The second approach is collecting the faeces after oral feeding of prebiotic (Gibson *et al.*, 2004). Each method has an advantages and limitation. Selection of the method was based on the purpose of the study and the availability of laboratory facility.

The objectives of this study were to analyze the macronutrients composition, dietary fiber, and resistant starch in Bestak sweet potato variety; to identify the prebiotic components in sweet potato fiber; and to evaluate the prebiotic activity of sweet potato fiber.

Materials and Methods

Bacterial strain

Lactobacillus plantarum Mut7 (FNCC 250), *Bifidobacterium longum* JCM 1217, and *Escherichia coli* FNCC 0091 were obtained from Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia. The bacteria were kept at -20 ^oC in 10% skim milk (w/v) and 10% glycerol (w/v). For the prebiotic activity assays, frozen cultures were streaked onto MRS agar (Merck) for *Lactobacillus plantarum* Mut7 and *Bifidobacterium longum* JCM 1217 cultures or Nutrient Broth (Merck) for *E. coli* FNCC 0091, followed by incubation at 37^oC for 24-48 h. Then, one colony from each plate was transferred into 10 mL of MRS broth (Merck) or Nutrient Broth and incubated overnight.

Prebiotic and sweet potato fiber preparation

The commercial prebiotics used in this study were fructooligosaccharide (FOS, Raftilose P95) and inulin (Fibruline 97%, Cosucra, Germany). Both of them were obtained from PT Alam Subur Tirta Kencana, Jakarta, Indonesia. Bestak sweet potato variety was obtained from Karanganyar, Central Java, Indonesia. The properties of the sweet potato were white color skin, 8 cm diameter, and 20-25 cm length. SPFE was prepared as follow: raw sweet potato was peeled, washed, and cut into cubes (2x2x2 cm^3). The sweet potato was then steamed for 30 min, extracted with ethanol 80% at 60^oC for 20 min. The mixture was filtered through linen filter cloth and the cake was dried at 50^oC for 24 h, ground and sieved with 80 mesh sieves. The prebiotic content of SPFE was determined by High Performance Liquid Chromatography (HPLC-Reflective Index Detector) using FOS, inulin, raffinose, and verbascose as standard. There were 4 different pre-treatment for SPFE powder before prebiotic analysis i.e. 1) SPFE powder without dilution and heat treatment (S_1); 2) SPFE powder was diluted with 10 parts of deionized water, without heated (S_2); 3) SPFE powder was diluted with 10 parts of deionized water and heated at 100^oC for 20 min (S_3); and 4) SPFE powder was diluted with 10 parts of deionized water and heated at 121^oC for 20 min (S_4). These pre-treatment were conducted based on our previous study that heat treatment of SPFE could increase the production of IgM in HB4C5 cell line (unpublished data). Briefly, 2.5 g sample was put into 25 mL conical tubes, added with 5 mL deionized water, mixed and added with 20 mL CH_3CN, mixed and then filtered through Whatman paper filter. The filtrate was evaporated with a rotary evaporator to evaporate out all the CH_3CN. The aqueous phase was dried with N_2 in a waterbath at 50^oC. After drying process, the sample was added with 1mL deionized water and filtered through Millex 0.45 µm. As much as 20 µL of filtrate was injected in HPLC column (Metacarb 87C). The column was eluted isocratically with deionized water at 85^oC and a flow rate of 0.7 mL/min.

Macronutrient analysis

The ash was determined by dry ashing method (Nielsen, 2003). Nitrogen content was determined using the Kjeldahl method and multiplied by a factor 6.25 to determine the protein content (Nielsen, 2003). The crude fat was determined by Soxhlet method (Nielsen, 2003), whereas the total carbohydrate was calculated with "by difference method". The starch was determined by Somogyi-Nelson method after degradation of starch into reducing sugar (Nielsen, 2003). The soluble and insoluble fibers were determined by enzymatic gravimetric method (Asp *et al*., 1983), whereas the resistant starch was determined by the method described by Goni *et al.* (1996). All results were expressed on a dry weight basis.

Prebiotic activity assay

Prebiotic activity reflects the ability of a given substrate to support the growth of an organism relative to other organisms and relative to growth of a non-prebiotic substrate such as glucose. The assay was performed by substitution of glucose with prebiotic in MRS broth medium. MRS broth contains 20 g/L glucose. An overnight culture of probiotic strain was inoculated into MRS broth containing 2% (w/v) glucose or prebiotic. The cultures were incubated at 37^oC under anaerobic condition for *B. longum* JCM 1217 and micro aerobic condition for *L. plantarum* Mut7. After 0 and 24 h of incubation,

samples were enumerated on MRS agar. In addition, overnight cultures of *E. coli* FNCC 0091 was added at 1% (v/v) to separate tubes containing M9 broth with 2% (w/v) glucose or prebiotic. The cultures were incubated at 37°C and enumerated on Tryptone Bile X-Glucuronide (TBX) agar after 0 and 24 h of incubation. Each assay was replicated three times.

Prebiotic activity score

The prebiotic activity score was determined using the equation described by Huebner *et al*. (2007).

Prebiotic activity score = {(probiotic log CFU mL^{-1} on the prebiotic at 24 h – probiotic log CFU mL^{-1} on the prebiotic at 0 h) / (probiotic log CFU mL^{-1} on glucose at 24 h – probiotic log CFU mL^{-1} on glucose at 0 h)} - {(enteric log CFU mL^{-1} on the prebiotic at 24 h – enteric log CFU mL^{-1} on the prebiotic at 0 h) / (enteric log CFU mL^{-1} on glucose at 24 h – enteric log CFU mL^{-1} on glucose at 0 h)}.

Statistical analysis

Each result is expressed as the mean ± standard deviation (SD). T-test was used to assess the statistical significance of differences. Each value of $p < 0.05$ or $p < 0.01$ is considered to be statistically significant.

Results and Discussions

Macronutrients of the Bestak sweet potato variety

Table 1 showed the macronutrients composition of Bestak sweet potato variety. The macronutrients composition of food can be expressed either as a wet basis percentage (g / 100 g food) or a dry basis percentage (g / 100 g dry solids). A dry basis percentage was better to compare the nutrient composition of raw and processed food sample. The results indicated that the protein content of SPFE was higher than in the raw and the powder. The raw sweet potato showed the lowest protein contents (2.42%), whereas the sweet potato fiber showed the highest protein contents (3.19%). The protein was determined by the Kjeldahl method. The Kjeldahl method has several disadvantage i.e.: 1) it measures total organic nitrogen, not just protein nitrogen, 2) the precision was poorer that the biuret method (Nielson, 2003). We assumed that the highest protein contents in SPFE might be due to the digestion process during dietary fiber extraction. Heat treatment and extraction using 80% ethanol will result in protein denaturation and subsequently release more amino acids and nitrogen. The ash, starch, and total carbohydrate in the SPFE were lower than in the raw and the powder. It might be due to the lost during extraction process of SPFE.

Figure 1 showed that the insoluble dietary fiber, resistant starch, and total dietary fiber in the SPFE

Table 1. Macronutrients composition of Bestak sweet potato variety

Component	Sweet Potato		
	Raw	Powder	Fiber Extract
Protein (%db)	2.42 ± 0.15	2.61 ± 0.07	3.19 ± 0.02*
Fat (%db)	0.71 ± 0.07	1.08 ± 0.09	1.08 ± 0.13
Ash (%db)	2.17 ± 0.02	1.95 ± 0.31	1.46 ± 0.00**
Starch (%db)	86.97 ± 3.10	75.02 ± 1.52*	62.86 ± 0.55**
Total Carbohydrate (%db)	94.70 ± 0.10	94.36 ± 0.29	94.27 ± 0.11*

Note: The results are expressed as means ± standard deviations for duplicate analysis. Statistically significant differences from raw sweet potato are represented as $^{*}p < 0.05$ or $^{**}p < 0.01$

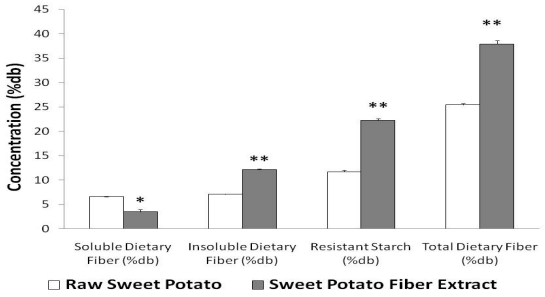

Figure 1. Dietary fiber composition of Bestak sweet potato variety

Note: Each bar represent average of each samples. The significantly differences from raw sweet potato are represented as $^{*}p < 0.05$ or $^{**}p < 0.01$.

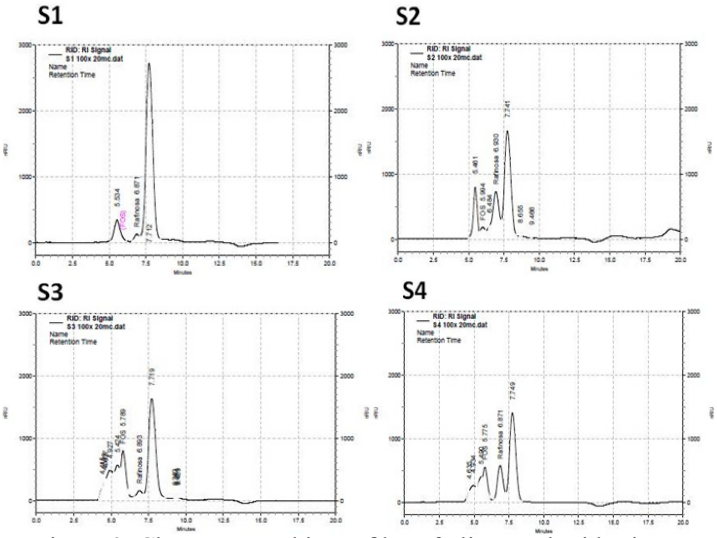

Figure 2. Chromatographic profiles of oligosaccharides in Sweet Potato Fiber Extract (SPFE)

Note: The prebiotic content of SPFE was analyzed by High Performance Liquid Chromatography (HPLC-Reflective Index Detector) using FOS, inulin, raffinose, and verbascose as standards. FOS and inulin standard injection gives the same result (the peak appears in the same time). The peak that represents verbascose did not appear in all samples.
S1: SPFE powder without dilution and heat treatment
S2: SPFE powder was diluted with 10 parts of deionized water, without heated
S3: SPFE powder was diluted with 10 parts of deionized water and heated at 100°C for 20 min
S4: SPFE powder was diluted with 10 parts of deionized water and heated at 121°C for 20 min.

was higher than in the raw sweet potato; however the soluble fiber was lower. The decreasing of the soluble dietary fiber was due to the lost during extraction process. According to Fadaei and Salehifar (2012), the chemical extraction of dietary fiber result in a fewer dietary fiber compare to enzymatic method, however chemical extraction is easier than enzymatic extraction.

Prebiotic content in sweet potato fiber

Figure 2 and 3 showed that sweet potato fiber

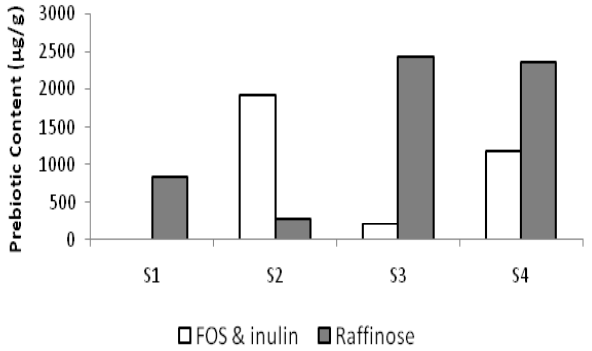

Figure 3. Prebiotic content of sweet potato fiber extract
Note: Each bar represent the value from single measurement of HPLC injection. The concentration of FOS and inulin in S1 sample was under the limit of detection (< 0.15 µg/g).
S1: SPFE powder without dilution and heat treatment
S2: SPFE powder was diluted with 10 parts of deionized water, without heated
S3: SPFE powder was diluted with 10 parts of deionized water and heated at 100°C for 20 min
S4: SPFE powder was diluted with 10 parts of deionized water and heated at 121°C for 20 min.

Table 2. Increasing the cell number after 24 h of incubation (reported as log10 CFU/mL)

Bacterial Culture	Glucose	SPFE	Inulin	FOS
L. plantarum Mut7	2.44 ± 1.28	3.21 ± 0.63	2.49 ± 0.40	2.23 ± 0.07
B. longum JCM1217	1.64 ± 0.13	1.99 ± 0.00	1.84 ± 0.10	2.19 ± 0.06*
E. coli	1.18 ± 0.27	1.08 ± 0.05	2.35 ± 0.08*	1.10 ± 0.19

Note: The results are expressed as means + standard deviations for triplicate analysis. The significantly differences from glucose for each bacteria are represented as $^{*}p < 0.05$ or $^{**}p < 0.01$.

extract contained FOS, inulin, and raffinose, whereas verbascose was not detected. Several other peaks were detected but these compounds were not identified yet. The prebiotic content of non-diluted SPFE powder was lower than diluted SPFE. Heat treatment could increase the raffinose of SPFE, but it could decrease the FOS content. The results from this study indicated that heat treatment of SPFE could change the concentration of prebiotic. Similar to this finding, Böhm *et al.* (2005) found that degradation of inulin was induced by heat treatment. Dry heating of inulin from chicory for up to 60 min at temperature between 135 and 195°C resulted in a significant degradation ranging from 20-100%. Inulin and FOS were similar except they were different in the degree of polymerization (DP). DP refers to the number of repeat units in an oligomer or polymer chain. The DP of inulin was ranging from 2-60, whereas the DP of FOS was less than 10.

Growth of L. plantarum Mut7, B. longum JCM 1217 and E. coli FNCC 091 on prebiotic carbohydrate

Increasing of the number of the cell was measured after incubation of 24 h. *L. plantarum* Mut7 and *B. longum* JCM 1217 were grown on 2% (w/v) glucose or prebiotic. Table 2 showed that the growth of *B. longum* JCM 1217 was better in the presence of FOS. *L. plantarum* Mut7 grew better in the presence of SPFE substrate, although the differences with any other substrates were not significant. This indicated that SPFE, inulin, and FOS were metabolized as well as glucose by *L. plantarum* Mut7. Prebiotic should be metabolized by bacterial culture as well as glucose is metabolized.

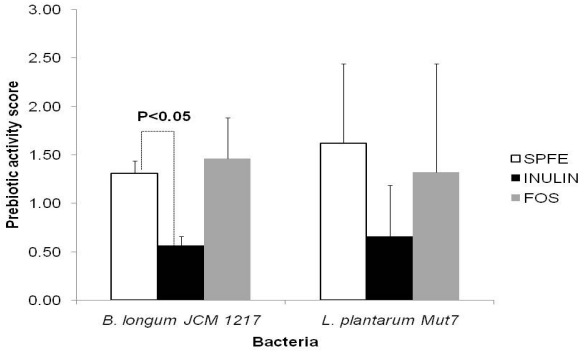

Figure 4. Prebiotic activity score of *B. longum* JCM 1217 and *L. plantarum* Mut7 grown in SPFE, inulin, and FOS substrate
Note: Each bar represent average of prebiotic activity score for *B. longum* JCM1217 or *L. plantarum* Mut7 grown in prebiotic substrates.

The important characteristic of prebiotic is that it should be selective and not fermented by commensal bacteria such as *E. coli.* The results from this study indicated that the increasing number of *E. coli* FNCC 091 grown on 2% (w/v) of SPFE and FOS were lower than that grown on 2% (w/v) of glucose but there were not significantly different. The increasing number of *E. coli* grown on 2% (w/v) of inulin was greater than on glucose. It is indicated that *E. coli* could ferment inulin better than glucose. Moongngarm *et al.* (2011) also found that *E. coli* could ferment inulin, FOS, and other type of prebiotic better than glucose. Although the definition of prebiotic as could be fermented by specific colonic bacteria especially lactic acid bacteria, it now appears that Bacteroides and other commensal microbiota in the GI tract have the metabolic capacity to metabolize prebiotic (Van der Meulen *et al*., 2006), Furthermore, if the lactic acid bacteria fermented oligosaccharides in the GI tract, it would release mono- or disaccharides that could be used by other organism in GI tract including *E. coli*.

Prebiotic assay activity

The prebiotic activity score was showed in Figure 4. The highest prebiotic activity scores was for *L. plantarum* Mut7 (1.62 ± 0.58) although it was not significantly different compared to *L. plantarum* Mut7 grown on inulin (0.66 ± 0.52) and FOS (1.32 ± 0.79). *B. longum* JCM 1217 grown on SPFE substrate shown a highest prebiotic activity score compared to inulin and FOS. The prebiotic activity score for *B.*

longum JCM 1217 and *L. plantarum* Mut7 grown on SPFE substrate were not significantly different. It was indicated that *L. plantarum* Mut7 could ferment SPFE as well as *B. longum* JCM 1217.

Due to its longer chain length, inulin is less soluble than FOS and therefore it was slowly fermented by specific bacteria compared to FOS. This study showed that inulin had lower prebiotic activity score compared to SPFE and FOS. These results were similar to that studied by Huebner *et al.* (2007) who found that *L. plantarum* 4008 and *L. plantarum* 12006 grown on inulin had a lower prebiotic activity score compared to those grown on Raftilose P95 (FOS in this study). We found that prebiotic activity score of SPFE was not significantly different with FOS for all probiotic bacteria tested. It is indicated that SPFE could be fermented by *L. plantarum* Mut7 and *B. longum* JCM 1217 as well as FOS.

Calculation of prebiotic activity score using Huebner's equation was simpler than other equation developed by Olano-Martin *et al.* (2002), Palframan *et al.* (2003), Vulevic *et al.* (2004), and Sanz *et al.* (2005 a, b). This method was simpler than others because it did not require fecal samples and relatively faster to evaluate the ability of prebiotic to be fermented by specific bacteria.

Conclusions

The SPFE extracted from Bestak sweet potato variety has potency as prebiotic source because it contains FOS, inulin, and raffinose. The prebiotic activity score of SPFE was similar to FOS, but it was higher than inulin.

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