Potential Chemopreventive Properties and Varietal Difference of Dietary Fiber from Sweetpotato (*Ipomoea batatas* L.) Root

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Abstract

The potential chemopreventive properties of the dietary fiber prepared from sweetpotato (*Ipomoea batatas* L.) roots were examined to promote the demand of this residue from the starch industry. Dietary fiber was prepared by treating starch granules-removed residue with α -amylase. The dietary fibers at 1% concentration enhanced the growth of *Bifidobacterium adolescentis* and *Bifidobacterium breve in vitro* among 5 species of *Bifidobacterium*, which exist in the human intestinal tract. Analysis of the components of these fibers suggested that their pectin and hemicellulose were concerned with a promotion effect on the growth of bifidobacteria. Water- and oil-holding capacity of the fibers in the varieties with orange-colored flesh was relatively superior to ones from the varieties with yellow- or purple-colored flesh. Furthermore, the dietary fibers adsorbed about 90% of the mutagen, Trp-P-1. Commercial sweetpotato fiber, a byproduct of citric acid production from the residue from the starch industry, had slight effect on the growth of bifidobacteria and was lower in adsorption capacity of Trp-P-1 than the fibers prepared from sweetpotato roots. These results indicate that the residue from the sweetpotato root starch industry is available as a dietary fiber with physiological functions.

Discipline: Food

Additional key woods: residue from starch industry, bifidobacteria, fiber component, water-holding capacity, oil-holding capacity

Introduction

In recent years new varieties of sweetpotato (*Ipo-moea batatas* L.) have been released for the development of new uses from the National Agricultural Research Center for Kyushu Okinawa Region (KONARC), Miya-konojo, Japan. New products, such as juice, powder, and brewed drinks, which are made from flesh-colored sweetpotatoes, have been made practicable²⁶. However, disposition of *shochu* waste, residue from the starch industry, and strained residue from juice production is a critical problem from the viewpoint of environmental protection. Residue from the sweetpotato starch industry has been used as a raw material in citric acid fermentation. However, utilization of this residue has become increasingly difficult, due to the import of foreign-made cheap citric acid. Therefore development of new uses for resi-

due from the starch industry has been demanded for a long time.

Substantial epidemiological and physiological studies have shown that dietary fiber has important functions in the diet, with suggestions that it gives protection against cardiovascular disease, colon cancer, and diabetes, the various fiber components having roles in this respect²³. Dietary fiber removes health-harmful factors, such as artificial food color²¹, aluminum²², mutagens^{1,9}, and cholesterol¹¹ by adsorption of these factors from the body and improves the flora of intestinal bacteria¹³. Dietary fibers have been used as functional foods for protection from colon cancer and heart disease in Western countries and to relieve constipation in Japan. Furthermore, dietary fiber has been actively used to decrease calories and improve food quality. Therefore, an expansion in the demand for dietary fibers is expected in the future. Studies on dietary fibers have been carried out for soy-

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bean, rice bran, wheat bran, corn skin, and vegetables². However, the physiological functions of sweetpotato dietary fibers have been little reported so far. Sweetpotato root has a well-balanced content of soluble and insoluble fiber in a ratio of about 1:1 (excluding lignin), unlike wheat bran, which is mainly insoluble fiber^{3,7}. This is to say, sweetpotato dietary fiber contains abundant soluble components, pectin or hemicellulose, suggesting that physiological functions may be higher in sweetpotato fibers than in other crops.

In this paper, we describe some potential chemopreventive properties and varietal difference of dietary fiber from sweetpotato root.

Materials and Methods

1. Bacteria

Bifidobacterium (B.) bifidum JCM1254, *B. infantis* JCM1222, *B. breve* JCM1192, *B. longum* JCM1217, *and B. adolescentis* JCM1275 were purchased from the Institute of Physical and Chemical Research, Tsukuba, Japan. Strain TA98 of *Salmonella typhimurium* was supplied by the Institute for Fermentation, Osaka, Japan (IFO).

2. Sweetpotato materials and preparation of dietary fibers

Various kinds of sweetpotato were cultivated in 2000 under the same conditions in an experimental field of KONARC at Miyakonojo, Japan. Preparation method of dietary fiber referred to the reports of Noda et al.¹⁶ and Takamine et al.²⁰. About 1 kg of sweetpotato storage root was washed, dice-cut, and homogenized by a mixer with distilled water. The homogenate was crushed by ultrafine friction grinder (Supermasscolloider, Masuko Sangyo Co., Ltd., Saitama, Japan) and put through a 200mesh sieve. The resultant starch fiber was dehydrated and dried for 24 h at 50°C. Crude starch fiber was suspended to 1 L of deionized water and the suspension was adjusted to pH 6.3. To this suspension 0.04 mL (120 U) of α -amylase solution (Speedase HS, Nagase Biochemical, Co., Kyoto) was added and digestion was carried out for 30 min at 95°C. After that, the suspension was cooled at 60°C and 0.04 mL (280 U) of glucoamylase solution (NEO XL-128, Nagase Biochemical, Co., Kyoto) was added and the reaction was incubated for 6 h at 60°C. Starch-removed fiber was collected through a 300-mesh sieve and washed thoroughly with deionized water. Resultant fiber was freeze-dried and powdered with a mill.

Commercial sweetpotato fiber, a residue after citric acid production of the residue from the starch industry, was received from Kyushu Kako Co., Kagoshima, Japan. This fiber was also freeze-dried, ground and used for the experiments.

3. Preparation of boiled water-soluble and -insoluble fraction

Fiber sample (1.5 g) was boiled in 30 mL of deionized water for 10 min. The suspension was centrifuged at $39,000 \times g$ for 10 min and the supernatant was obtained. The above procedure for the precipitate was repeated once and the supernatants were finally mixed and lyophilized. The resultant insoluble fraction was directly lyophilized and powdered with a mill.

4. Fractionation of cell wall materials

Fiber components were fractionated according to the method of Shibuya and Iwasaki¹⁹. Fiber (1.5 g) was treated with 300 mL of 0.25% $(NH_4)_2C_2O_4$ solution at 90°C for 3 h. The mixture was filtered with a G-3 glass filter, and the filtrate was dialyzed against distilled water and freeze-dried to obtain the pectin fraction, while the residue was washed successively with distilled water, methanol, and acetone and air-dried. This residue was then treated with 4 M KOH containing 0.1% NaBH₄, incubated at room temperature for 24 h, and filtered. The alkaline extract was neutralized with CH₃COOH, dialyzed against distilled water, and freeze-dried to give the hemicellulose fraction. The residue was again washed with distilled water, methanol, and acetone and air-dried to give the α -cellulose fraction.

5. Adsorption of Trp-P-1 by the fiber

Fiber sample (20 mg/mL) and Trp-P-1 (25 μ g/mL, 3-amino-1,4-dimethyl-5*H*-pyrido-(4,3-*b*)indol, Wako Pure Chemical Industries Ltd., Osaka Japan) were mixed in 5 mL of 10 mM potassium phosphate buffer (pH 7.0) and incubated at 37°C for 1 h. After that, the mixture was centrifuged at 1,500 × g for 10 min and the supernatant was obtained. The precipitate was mixed with the same volume of the buffer and the remaining Trp-P-1 was re-extracted by the same procedure. The combined supernatants were diluted to 33 times the initial volume for the mutagenicity assay and the mutagenic activity was evaluated by the Ames method using *Salmonella typhimurium* TA98²⁵.

6. Measurement of bifidobacterial growth

Bifidobacteria were precultured in MRS medium (Oxoid Ltd., Hampshire, England) with the anaerobic jar, Gas Pack (Oxoid Ltd., Hampshire, England) at 37°C for 72 h. Effect of the fiber on bifidobacterial growth was determined in Peptone-Yeast-Fildes (PYF) medium⁸. PYF medium consists of 10 g Trypticase Peptone (BBL,

Sample	Growth of <i>Bifidobacterium</i> ^{a)}				
	B. adolescentis	B. bifidum	B. breve	B. infantis	B. longum
Koganesengan	++	_	++	_	_
J-Red	_	_	±	_	±
Kyushu 124	++	_	++	±	_
Ayamurasaki	++	_	++	_	±
Kokei 14	+	_	+	_	_
Konahomare	++	_	++	_	_
Commercial SP fiber	_	_	-	_	_
Glucose	+++	+++	+++	+++	+++

Table 1. Effect of sweetpotato fiber on growth of Bifidobacterium

a): Bacterial fermentation was evaluated by the pH of the broth (the mean of three experiments) after cultivation. -: 6<pH, ±: 5.5<pH<6.0, +: 5.0<pH<5.5, ++: 4.5<pH<5.0, +++: pH<4.5.

Becton Dickinson & Company, Cockeysville, MD, USA), 5 g yeast extract (Difco Lab., Detroit, MI, USA), 0.5 g L-cysteine hydrochloride, 40 mL digested horse blood, and 40 mL salt solution per 1 L. Salt solution contains 0.2 g CaCl₂, 0.2 g MgSO₄·7H₂0, 1.0 g KH₂PO₄, 1.0 g K₂HPO₄, 10 g NaHCO₃, and 2.0 g NaCl in 1 L deionized water (pH 7.6). Fiber sample was added to PYF medium of 10 mL at a final concentration of 1% and glucose was added as a positive control at the same concentration. The medium containing the fiber sample or glucose was autoclaved and cooled at room temperature. Precultured Bifidobacterium suspension of 10 µL was added to the medium containing the sample or glucose and was incubated with the Gas pack at 37°C for 72 h. The growth of bacteria was evaluated by the lowering of pH, which was caused by acid production with Bifidobacterium growth.

7. Measurement of water- and oil-holding capacity

Water- and oil-holding capacity of the fibers was measured according to a modification of the centrifugation method of McConnell et al.¹². The fiber sample of 0.3 g was weighed precisely in a 50 mL centrifugal tube and rapeseed oil (Nacalai Tesque, Inc., Kyoto, Japan) or water was added in order for the tube contents to weigh 30 g. The mixture was suspended with the Polytron homogenizer (PT-MR 3000, Kinematica AG, Littau, Switzerland) at 12,000 rpm for 10 sec. The suspension was centrifuged at 46, 417, 1,670, and 4,100 × g for 30 min and the resultant precipitate was weighed. Waterand oil-holding capacities were shown by the weight of the water or oil that 1 g of dry fiber could hold.

Results

1. Effect of sweetpotato fiber on the growth of *Bifidobacterium*

The results of fermentation of the fibers from 6 varieties, Koganesengan (yellow-colored flesh), J-Red (orange-colored flesh), Kyushu 124 (yellow-colored flesh), Ayamurasaki (purple-colored flesh), Kokei 14 (yellow-colored flesh), Konahomare (yellow-colored flesh), commercial sweetpotato fiber, and glucose are shown in Table 1. Fiber sample and glucose were added to the medium at a concentration of 1%. Fermentation of the materials by the bifidobacteria was evaluated by measuring the pH of the incubated media. The pH was scored in the following manner: -, $6 \le pH$; \pm , $5.5 \le pH \le 6.0$; +, 5.0<pH<5.5; ++, 4.5<pH<5.0; +++, pH<4.5. Commercial sweetpotato and J-Red fiber had no effect on the growth of the 5 species of Bifidobacterium. B. adolescentis and B. breve fermented effectively Koganesengan, Kyushu 124, Ayamurasaki, and Konahomare fibers, and glucose. Kokei 14 fiber was slightly fermented by B. adolescentis and B. breve. These results suggest that sweetpotato fibers are fermented by limited numbers of bifidobacteria and furthermore there is a varietal difference of the sweetpotato dietary fiber on the promoting effect.

Pectin substance is solubilized by steaming or boiling of the fiber^{10,22}. Accordingly Ayamurasaki fiber was fractionated to water-soluble and -insoluble fractions by boiling. Fermentation of these fractions and non-treated fiber by *B. adolescentis* was compared (Table 2). Each sample was added to the medium at a final concentration of 1%. *B. adolescentis* effectively fermented the boiling water-soluble fraction as well as glucose, while the boiling water-insoluble fraction had no effect on *B. adolescentis* fermentation. Growth-promoting effect was higher in boiling water-soluble fraction than Ayamurasaki fiber itself.

2. Content of pectin, hemicellulose, and cellulose of sweetpotato and commercial fiber

Content of pectin, hemicellulose, and cellulose from the fibers from 6 varieties, Koganesengan, J-Red, Kyushu 124, Ayamurasaki, Kokei 14, Konahomare, and commercial sweetpotato fiber are shown in Table 3. Pectin, hemicellulose, and cellulose content varied 167–366 mg/g dry weight (DW), 89–397 mg/g DW, and 199–600 mg/g DW, respectively. These results suggest a varietal difference between the contents of pectin, hemicellulose, and cellulose. Pectin, hemicellulose, and cellulose contents of the commercial sweetpotato fiber were 44 mg/g DW, 121 mg/g DW, and 639 mg/g DW, respectively. Pectin and hemicellulose contents were much less in the commercial sweetpotato fiber than those prepared from 6 kinds of varieties.

3. Relationship between fiber components and bifidobacterial fermentation

The correlation between *B. adolescentis* growth and the fiber components was evaluated by the pH lowering of three days-fermented medium in order to clarify the relationship between the growth-promoting activity and fiber components (Table 4). Pectin (r = -0.665) and hemicellulose (r = -0.489) contents correlated recipro-

 Table 2. Effect of supernatant or precipitate centrifuged after boiling of Ayamurasaki fiber on growth of *B. adolescentis*

Sample	Growth of <i>B. adolescentis</i> ^{a)}
Non-treatment fiber	++
Supernatant of boiled fiber	+++
Precipitate of boiled fiber	_
Addition of glucose	+++
Non addition of glucose	-

a): Bacterial fermentation was evaluated by the pH of the broth (the mean of three experiments) after cultivation.

-: 6<pH, ±: 5.5<pH<6.0, +: 5.0<pH<5.5, ++: 4.5<pH<5.0, +++: pH<4.5.

Table 3.	Varietal difference of pec	tin, hemicellulose,	, and cellulose co	ontents from
	sweetpotato fiber			

Fiber sample	Сс	ntent of fiber componen (mg/g fiber DW)	nts ^{a)}
	Pectin	Hemicellulose	Cellulose
Koganesengan	167	397	199
J-Red	215	89	600
Kyushu 124	366	109	293
Ayamurasaki	353	135	275
Kokei 14	240	238	368
Konahomare	195	323	402
Commercial SP fiber	44	121	639

a): Content of pectin, hemicellulose, and cellulose are the means of three experiments.

 Table 4. Correlation coefficient between the pH of the medium after three-days fermentation and the content of the fiber components

Fiber component	Correlation coefficient (r)	
Pectin	- 0.665	
Hemicellulose	-0.489	
Pectin plus hemicellulose	- 0.934	
Cellulose	0.923	

=

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cally with the lowering of the pH in the medium after three days-fermentation. Furthermore correlation coefficient between total contents of pectin and hemicellulose and the pH values was -0.934. The correlation of cellulose (r = 0.923) content with the decrease of the pH was positive. These results suggest that both pectin and hemicellulose promote the growth of *B. adolescentis* and *B. breve*. The correlation of pectin content was higher than that of the hemicellulose, indicating that pectin is more effectively concerned with the growth-promoting effect of the bifidobacteria than hemicellulose.

4. Water- and oil-holding capacity of sweetpotato fiber

Correlation of water-holding capacity with oil-holding capacity of sweetpotato fiber (n = 18) and commercial fiber is shown in Fig. 1. Flesh color differed by variety and line. Nine varieties with orange-colored flesh, 5 varieties with purple-colored flesh, and 4 varieties with yellow-colored flesh, were used for this experiment. Water-holding capacity correlated with oil-holding capacity (r = 0.636). Oil-holding capacity of the fibers from the varieties with purple- and yellow-colored flesh was about 7-16 g/g DW, while oil-holding capacity of the fibers from the varieties with orange-colored flesh was about 19-25 g/g DW. Water-holding capacity of the fibers from the varieties with yellow- and orange-colored flesh was about 17-25 g/g DW and about 30-43 g/g DW, respectively. Further, water-holding capacity of the fibers from the varieties with purple-colored flesh was about 31-51 g/g DW except for Ayamurasaki (about 10 g/g DW). Water- and oil-holding capacities of the commercial sweetpotato fiber were the same degree as Ayamurasaki. Water- and oil-holding capacity of the fibers from the varieties with orange-colored flesh was relatively superior to ones from yellow- or purple-colored flesh. A variety with an excellent capacity of water holding was observed in those with purple-colored flesh.

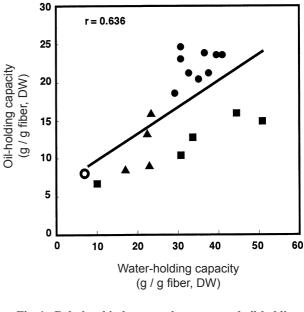


Fig. 1. Relationship between the water- and oil-holding capacity of sweetpotato fiber



5. Adsorption of Trp-P-1 by sweetpotato fiber

Trp-P-1 is a mutagenic pyrolysate of tryptophan, which was first isolated from broiled beef and demonstrated to be carcinogenic in rats¹⁵. Adsorption of Trp-P-1 by the sweetpotato fiber was determined by the Ames method of the mutagen remaining in the resultant supernatant after the centrifugation of the mixture of the mutagen and the fiber. Adsorption rate of Trp-P-1 by Shiroyutaka, Koganesengan, Kyushu 124, and the commercial sweetpotato fiber is shown in Table 5. Shiroyutaka, Koganesengan, and Kyushu 124 fiber adsorbed 87– 89% of the added mutagen, while the commercial sweetpotato fiber adsorbed 56%.

Sample	His ⁺ revertants /plate ^{a)}	Adsorption rate (%) ^{b)}	
None	$1,036 \pm 36$	0	
Shiroyutaka	132 ± 4	87	
Koganesengan	112 ± 6	89	
Kyushu 124	112 ± 18	89	
Commercial sweetpotato fiber	459 ± 60	56	

Table 5. Adsorption of Trp-P-1 to sweetpotato fiber

a): Each value represents the mean ± S.D. of triplicate plates. The values shown have had the spontaneous mutation frequency subtracted.

b): Adsorption rate (%) = $(1 - y/x) \times 100$.

y: Number of His⁺ revertants /plate at no addition of the fiber.

x: Number of His⁺ revertants /plate at addition of the fiber.

Discussion

Potential chemopreventive properties and varietal difference of dietary fiber from sweetpotato roots were examined to develop new uses of the residue from the starch industry. A predominantly bifidobacterial flora is considered to inhibit the growth of harmful bacteria such as pathogenic strains and protect infants against gastrointestinal diseases¹⁴. Consequently, factors that stimulate the growth of bifidobacteria appear to be promising effective substances for the maintenance of intestinal homeostrains. Sweetpotato fibers promoted effectively the growth of B. adolescentis and B. breve among 5 species of Bifidobacterium which exist in the human intestinal tract (Table 1). Boiling-soluble fraction of the Ayamurasaki fiber promoted more effectively the growth of B. adolescentis than the fiber itself (Table 2), suggesting that pectin may be the promoting factor of bifidobac-This is also consistent with the relationship teria between the fiber components and the growth promotion of bifidobacteria (Tables 3 & 4). A recent study demonstrated that among the root crops, sweetpotato cell wall material had the highest amount of the pectin fraction¹⁸. These data indicate that the sweetpotato fiber is available as physiologically functional material.

The water-holding capacity of dietary fiber is thought to be an important determinant of faecal bulking and intestinal transit times with influence on gastrointestinal disease^{4,6,24}. The present data showed that sweetpotato fiber had excellent capacities and these depended on the varieties. Takamine et al. indicated that the waterand oil-holding capacity of the dietary fiber made from residue of the sweetpotato root starch industry was superior to some commercial dietary fiber, corn and beet fiber²⁰. High oil-holding capacity means various kinds of mutagens and cholesterol can be adsorbed effectively, because most of these components are lipophilic. Sweetpotato fiber is by far the most effective binder of cholesterol at 30%, cassava fraction at 3%, citrus pectin at 8% and the majority of samples at <20% for 28 fiber samples from a variety of commonly consumed tropical fruits and vegetables including sweetpotato¹¹. It has been suggested that pectin with high methoxyl content is important in reducing serum cholesterol¹⁴. The methoxyl content of sweetpotato pectin was high at 9.7% of a cold water extract, the highest being for onion at 11% and wheat bran having only 0.1% in a study with a series of fruits and vegetables⁵. These reports reveal that sweetpotato dietary fiber is an effective agent for lowering cholesterol levels.

Some types of dietary fiber adsorb mutagenic agents¹⁷, which would lead to their excretion in the feces

and decrease their contact with colonic mucosal cells. Shiroyutaka, Koganesengan, and Kyushu 124 fiber adsorbed about 90% of the added mutagen, while the commercial sweetpotato fiber adsorbed 56%. Lower adsorption capacity of the commercial sweetpotato fiber seems to be caused by low content of pectin and hemicellulose. This is also supported by the report that cotton fiber containing cellulose as a main component slightly adsorbed Trp-P-1¹.

Commercial sweetpotato dietary fiber has been made mainly from residue from the starch industry of Koganesengan roots through citric acid production. However, the commercial sweetpotato fiber was relatively much lower in physiological functions than that prepared directory from Koganesengan roots. These results agreed well with the report of Salvador et al. that although commercial sweetpotato fiber was mainly composed of the cellulose fraction, small but significant amounts of the pectin and hemicellulose fractions were present as well¹⁸. Commercial sweetpotato fiber is prepared by treatment of the residue, after extraction of citric acid, with 0.25% NaOH and subsequent bleaching with NaOCl. Citric acid fermentation and this treatment seem to result in a decrease of pectin or hemicellulose in the commercial sweetpotato fiber and this thinking is supported by the component comparison between the commercial sweetpotato fiber and that of Koganesengan (Table 3).

In conclusion, our data showed that residue from the sweetpotato root starch industry was available as dietary fiber with physiological functions. However, the fiber used in this experiment was lyophilized samples and therefore the physiological functions were higher. Takamine et al. has revealed that heat drying of the sweetpotato fiber extremely lowers water- and oil-holding capacity²⁰. In the future the development of a drying method for the dietary fiber is necessary.

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