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Characterization of Soluble Dietary Fibers from Wax Gourd (*Benincasa hispida*) Pulp and Peel

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Abstract The physicochemical and *in vitro* physiological properties of soluble dietary fiber (SDF) from wax gourd (*Benincasa hispida*) pulp and peel were investigated. The pulp was composed of 11.4% SDF and 24.3% insoluble dietary fiber (IDF), while the peel contained 3.2% SDF and 43.3% IDF. The predominant sugar in the SDF of the wax gourd pulp and peel was uronic acid, followed by galactose and rhamnose. The SDFs from the wax gourd pulp and peel gave similar elution patterns, with 4 main neutral sugar and uronic acid peaks eluted by 0.4, 0.5, 1, and 2 M ammonium acetate buffer. The pulp SDF had a much higher glucose retardation index (GRI) than the peel SDF for all measurement times. The pulp SDF showed strong growth-inhibiting activity against *Escherichia coli* and *Clostridium perfringens*, whereas the peel SDF produced strong growth-promoting activity against *Bifidobacterium longum*, *Bifidobacterium infantis*, and *Lactobacillus brevis* when compared to glucose.

Keywords: soluble dietary fiber, wax gourd, glucose retardation effect, growth-inhibiting activity, growth-promoting activity

Introduction

Dietary fiber (DF) is defined as the plant components that are not digested by human gastrointestinal enzymes (1), and divided into soluble and insoluble fractions based on its solubility in aqueous medium. Fruits and vegetables are rich in soluble dietary fiber (SDF), whereas insoluble dietary fiber (IDF) is found in most cereals and legumes (2). These two types of fibers have received considerable attention due to their beneficial effects on constipation, coronary heart disease, obesity, diabetes, colonic cancers, and gastrointestinal disorders (3-5). In addition, DFs from fruits and vegetables have been reported to lower postprandial serum glucose and cholesterol levels, and stimulate the growth of a number of beneficial bacteria in the colon (6-8). The increased popularity of fiber-enriched health foods has resulted in the development of additional DF products containing biological benefits.

Benincasa hispida (Thunb.) Cogn., which belongs to the family of Cucurbitaceae, is native to tropical Asia and is mainly grown in India and China (9,10). It is commonly known as wax gourd, ash gourd, or white gourd in English (11). The wax gourd has been used as a food and folk medicine for a long time in Asian countries to treat gastrointestinal problems, respiratory diseases, heart diseases, vermifuge, diabetes mellitus, urinary diseases, hypertension, and inflammation (12,13). Recent research has shown that wax gourd possesses anti-angiogenic and antiulcer effects, and prevents constipation and hypercholesterolemia (11,14,15). The wax gourd's influence on physiological function might be related to the large amount of DF it contains (16). In particular, alcohol insoluble residues from fruit contain large amounts of pectic substances (9), suggesting that it could be a potential source of DF and pectin.

In this study, some physicochemical and *in vitro* physiological properties of SDFs from wax gourd pulp and peel were examined to enhance their usage value as a DF source. SDFs from the pulp and peel were extracted, and their carbohydrate composition and chromatographic characteristics were investigated. Furthermore, *in vitro* studies on the SDF hypoglycemic effects and growth effects on intestinal bacteria were performed.

Materials and Methods

Materials The wax gourd fruits were purchased from farmhouse (Gimjae, Jeonbuk, Korea), and cut into small pieces to remove the outer skin and core. The fruit was separated into pulp and peel, and then freeze-dried. The dried samples were ground with a blender (FM 680T; Hanil, Seoul, Korea) to pass through a 100 mesh sieve, and stored in a deep freezer at −20°C until use.

Chemical analysis Moisture, crude lipid, crude protein, and crude ash contents were determined by the AOAC official methods (17). DF content was determined using the AOAC official method after enzymatic removal of starch and protein (18).

Preparation of SDF The SDFs from the wax gourd pulp and peel were prepared using a DF kit (Sigma-Aldrich Co., St. Louis, MO, USA). Samples (1 g) dispersed in phosphate buffer (0.08 M, pH 6.0, 50 mL) were treated sequentially with α-amylase (100°C, 30 min), protease (60°C, 30 min), and amyloglucosidase (60°C, 30 min), and then filtered with sintered glass filters (1G3, Pyrex; Iwaki Glass Co.,

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Tokyo, Japan). The filtrate was precipitated with 4 volumes of ethanol (60°C, 95% v/v) for 1 day and then centrifuged at 6,500×g. The residues were washed out with 78% ethanol (20 mL), 95% ethanol (20 mL), and finally acetone (20 mL) after the supernatant was removed. The residues were then air-dried overnight at 40°C. Water was added to the dried residue to make a 2% solution. The solution was filtered with filter paper (No. 6, Advantec, Toyo Roshi Kaisha Ltd., Tokyo, Japan), and then freeze-dried.

Analysis of total uronic acid and individual sugars Total uronic aid content was measured using the *m*-hydroxydiphenyl method (19), and glucuronic acid and galacuronic acid were used as the standard materials for analyzing uronic acid. The individual sugars were analysed by a Bio-LC (DX 500; Dionex Co., Sunnyvale, CA, USA) fitted with a gradient pump (Dionex Co.), PA1 column (Dionex Co.), and pulsed amperometric detector (Dionex Co.) (20). The SDF was hydrolyzed by 12 M sulphuric acid at 35°C for 1 hr and then boiled in 2 M sulphuric acid at 100°C for 1 hr for further hydrolysis. After hydrolysis, the solution was diluted to make up to 25 mL using distilled water, and used in the analysis of individual sugars. The samples were diluted to 12.5 times, and filtered through a 0.45 μm membrane filter (Syringe filter, Wiesbaden, Germany) prior to injection.

Gel filtration and ion exchange chromatography Gel filtration chromatography (GE Healthcare Bio-science AB, Uppsala, Sweden) was performed on a XK column (2.6×100 cm) packed with Sepharose CL-6B (21). The column was eluted with 0.1 M Na-phosphate buffer (pH 6.0) at 0.4 mL/min. Samples (5 mg) were solubilized in 10 mL of 0.1 M Na-phosphate buffer (pH 6.0), and the supernatants (2 mL) from aqueous portions were applied on the column at a flow rate of 0.4 mL/min. Fractions (5 mL) were collected to analyze total sugar content using the phenol-sulfuric acid method (22). Dextran 2,000 kDa, 500 kDa, 70 kDa, 10 kDa (Sigma-Aldrich Co.), and glucose were used as molecular weight markers.

Ion exchange chromatography (GE Healthcare Bio-science AB) was performed on a XK column (2.6×30 cm) packed with diethyl aminoethyl (DEAE) Sepharose CL-6B (23). The column was eluted with 0.05 M ammonium acetate buffer, followed by a linear gradient (0.1 to 3 M) of ammonium acetate buffer (pH 6.0). The samples (72 mg) were solubilized in 12 mL of distilled water, and supernatants (2 mL) from the aqueous portions were applied on the column at a flow rate of 1 mL/min. Fractions (10 mL) were collected to analyze the neutral sugar and uronic acid contents.

Retarding effect on glucose transport The retarding effect of the SDF from wax gourd pulp and peel on *in vitro* glucose transport was measured using the procedure described by Adiotomre *et al.* (7). The glucose retardation effect was expressed as:

$$\text{Glucose retardation index (GRI, \%)} = \left(1 - \frac{\text{Total glucose diffused from sack containing fiber}}{\text{Total glucose diffused from sack without fiber}}\right) \times 100$$

Bioavailability on the growth of intestinal bacteria Six bacterial strains (*Escherichia coli* KCTC 2441, *Clostridium perfringens* KCTC 3269, *Lactobacillus brevis* KCTC 3102, *Bifidobacterium infantis* KCTC 3127, *Bifidobacterium bifidum* KCTC 3202, and *Bifidobacterium longum* KCTC 3128) were activated in brain heart infusion (BHI) and MRS broth medium and used to study the wax gourd pulp and peel SDF bioavailability for intestinal bacteria. The activated strains were cultivated anaerobically using an AnaeroGemTM pack (Oxoid Ltd., Basingstoke, England) and Anaero Jar (Oxoid Ltd.) at 37°C for 48 hr (24). Bacterial growth was measured using the method of Lee and Kim (25), with a slight modification. The samples (1, 3, and 5 mg) were added to reinforced clostridial medium (RCM), incubated anaerobically at 37°C for 48 hr using the AnaeroGemTM pack (Oxoid Ltd.) and Anaero Jar (Oxoid Ltd.), and then bacterial growth was measured spectrophotometically at 600 nm. A growth promoting response was expressed as the growth increase rate [GIR=(A_{600} sample $-A_{600}$ bacteria)/A_{600} control] (24). The experimental group was composed of 9.7 mL of RCM, 100 μL of bacteria, and 200 μL of sample. The bacteria control group was composed of 9.7 mL of RCM, 100 μL of bacteria, and 200 μL of distilled water, while the sample control group contained 9.7 mL of RCM, 100 μL of distilled water, and 200 μL of sample.

Results and Discussion

Proximate composition of wax gourd pulp and peel The proximate compositions of the wax gourd pulp and peel are given in Table 1. The pulp had higher amounts of crude protein and crude ash, but much lower crude fat and Klason lignin content as compared to the peel. The total dietary fiber (TDF) contents (46.46%) obtained from this study were higher than those found in persimmon peel (40.35%), and in edible portions (9.4-28.8%) of apple, pear, peach, and persimmon; however, the TDF levels were lower than those obtained from the pulp and peel (48.5-49.4%) of guava, and the alcohol insoluble polysaccharide (50.3%) from cucumber peel (23,27-29).

The main fraction of both the pulp and peel was IDF. However, the SDF content (11.39%) of the pulp was much higher than that of the peel (3.18%), indicating that wax gourd pulp could be a good source of SDF. In addition, the IDF/SDF ratio of the pulp (2.1:1), which should be within the range of 1.0-2.3 in order to have the beneficial health

Table 1. Proximate composition of wax gourd pulp and peel (%, dry basis)

Component	Pulp	Peel
Crude protein	14.72±0.67[1)]	13.38±0.37
Crude fat	1.46±0.02	4.37±0.53
Crude ash	12.76±0.52	8.26±0.62
Klason lignin	3.76±0.12	16.08±0.18
Total dietary fiber	35.66±1.07	46.46±0.89
Soluble dietary fiber	11.39±0.83	3.18±0.30
Insoluble dietary fiber	24.27±0.24	43.28±0.59

[1)]Determined in duplicate dry samples (mean±SD).

Table 2. Monosaccharide composition of SDFs from wax gourd pulp and peel[1)]

	Carbohydrates[2)] (%)							Total sugar (mg/g)
	Fuc	Rha	Ara	Gal	Glc	Xyl/Man	UA	
Pulp	2.31	4.77	3.95	26.95	4.09	0.97	56.97	222.99
Peel	1.68	6.66	4.38	13.14	4.38	0.25	69.52	356.31

[1)]Data expressed as percentages of total sugar.
[2)]Fuc, fucose; Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; Man, mannose; UA, uronic acid.

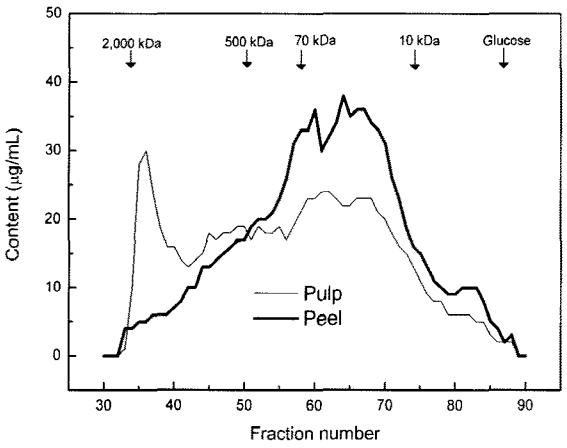

Fig. 1. Gel filtration chromatograms of SDFs from wax gourd pulp and peel.

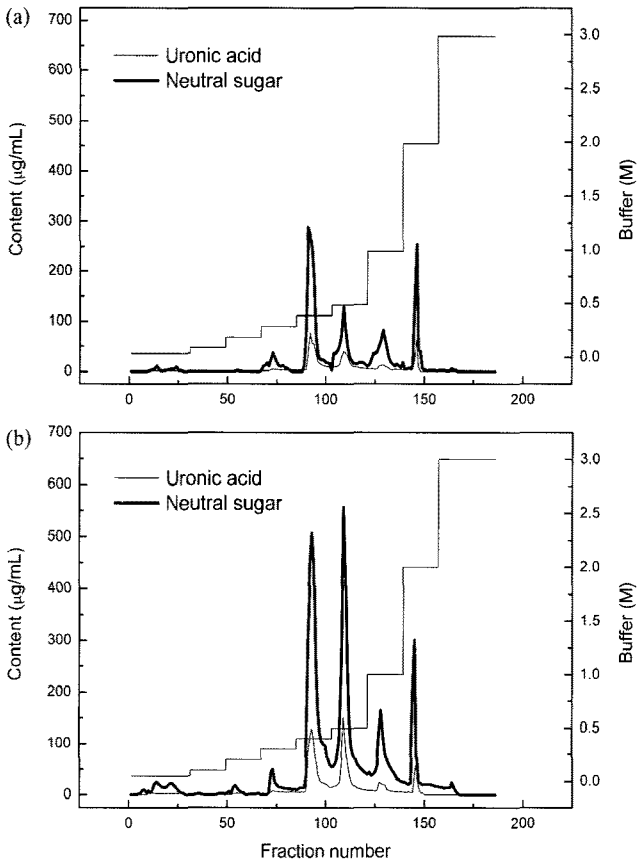

Fig. 2. Ionic exchange chromatograms of SDFs from wax gourd. (a) Pulp, (b) peel. The column was eluted with 0.05-3 M linear gradient of ammonium acetate buffer (pH 6.0).

effects associated with SDF and IDF (2,30), was lower than that of the peel (13.5:1), suggesting that the pulp powder from wax gourd is a well-balanced DF.

Total uronic acid content and sugar composition of SDFs from wax gourd powder The peel SDF contained a much higher uronic acid content than the pulp SDF (Table 2), indicating that the peel was more enriched in ionically and covalently bound pectins compared to the pulp SDF (31). The SDFs from the wax gourd pulp and peel contained fucose, rhamnose, arabinose, galactose, glucose, xylose, and mannose. The predominant sugar in the wax gourd pulp and peel SDF was uronic acid (56.97 and 69.52%, respectively), followed by galactose (26.95 and 13.14%, respectively), and rhamnose (4.77 and 6.66%, respectively). The percentages of the 3 major components were 88.69 and 89.32% of the total sugar content of the SDF in the pulp and peel, respectively, suggesting that galactose-rich pectic substances could be a major component of wax gourd pulp and peel SDF. In particular, relatively high rhamnose content in the wax gourd pulp and peel SDF might indicate the presence of rhamnogalacturonan. These results confirmed the previous reports that the major component of SDF from fruit and vegetables was pectic substances containing mainly arabinose, galactose, and uronic acid (32-34).

Gel filtration and ion exchange chromatography of SDF The SDFs extracted from the wax gourd pulp and peel were applied to gel filtration chromatography, and their molecular weight distributions are shown in Fig. 1. The pulp SDF gave a broad profile with one sharp peak of high molecular weight close to 2,000 kDa; whereas the peel SDF showed 2 peaks with molecular weights between 70 and 10 kDa, and one peak of low molecular weight below 10 kDa. In particular, the pulp SDF had a higher proportion (approximately 38%) of high molecular weight distribution, ranging from 2,000 to 500 kDa, and a lower proportion (approximately 50%) of low molecular weight distribution, ranging less than 70 kDa, than those (approximately 20 and 66%, respectively) of the peel SDF.

The SDFs extracted from the wax gourd pulp and peel were also applied to ion exchange chromatography on DEAE sepharose CL-6B using a buffer gradient; their elution patterns are shown in Fig. 2. The wax gourd pulp and peel SDFs gave similar elution patterns, resulting in 4 neutral sugar and uronic acid peaks. The major sugar and uronic acid peaks of the pulp and peel SDFs were eluted by 0.4, 0.5, 1, and 2 M ammonium acetate buffer, and the minor peaks by 0.05 and 0.3 M ammonium acetate buffer. The peel SDF had higher amounts of neutral sugar and

Table 3. Glucose retardation effects of SDFs from wax gourd pulp and peel

	Glucose retardation index (%)				
	1 hr	2 hr	4 hr	8 hr	24 hr
Pulp	18.3	15.9	16.1	6.3	5.3
Peel	13.5	9.5	12.7	3.6	3.5

uronic acid in all major fractions as compared to the pulp SDF. Principally, the neutral sugar and uronic acid peaks of the peel SDF, eluted by 0.5 M ammonium acetate buffer, had the highest proportion (30.88 and 34.11%, respectively), whereas the pulp SDF had 19.73 and 28.17%, respectively.

Retarding effect of SDF on glucose transport The results of the wax gourd pulp and peel SDF's *in vitro* glucose retardation effects are shown in Table 3. The glucose retardation indices (GRIs) of the pulp and peel SDFs, which could be used as a preceding test for predicting the physiological effect of DF (35), were from 13.5-18.3 and 3.6-6.3 as the time increased from 1 to 8 hr. These values were close to those of Korean cabbage and apple pectin (35). The pulp SDF had a much higher GRI than the peel SDF for all measurement times, including 1, 2, 4, and 8 hr. The high glucose retardation effect of the pulp SDF might be due to both a higher proportion of high molecular weight distribution and lower proportion of low molecular weight distribution as compared to the peel SDF.

The high viscosity of SDF is reported to be the most effective attribute to retard glucose diffusion due to the trapping of glucose within the gel matrix formed by fibers, resulting in a lowering of postprandial serum glucose (7,24,35). In addition to the high viscosity of soluble fiber, a reduction of serum glucose could be obtained by other factors. Ou *et al.* (36) demonstrated that DFs could lower postpradial serum glucose levels by retarding glucose diffusion, by increasing the viscosity of the small intestine juices, preventing glucose diffusion by decreasing the concentration of glucose available in the small intestine, and by inhibiting α-amylase action by capsuling the starch and enzyme.

Concentration effect of SDF on the growth of intestinal bacteria The growth promoting and inhibiting activities of the SDFs from wax gourd pulp and peel on human intestinal bacteria are shown in Table 4, and are compared with glucose as a control. The bioavailability on the growth of intestinal bacteria was in the order of glucose>peel> pulp. The higher bioavailability of the peel SDF as compared to the pulp SDF might be due to a higher proportion of low molecular weight carbohydrates, as well as higher amounts of total sugar and uronic acid in the peel SDF. Glucose showed the strongest growth-promoting activity against beneficial intestinal microorganisms such as *B. longum*, *B. infantis*, *B. bifidum*, and *L. brevis*, whereas it showed strong growth-promoting activity against harmful intestinal microorganisms such as *E. coli* and *C. perfringens*. The pulp SDF showed moderate growth-promoting activity against *B. longum*, *B. infantis*, and *B. bifidum* at a 5 mg addition group, whereas the peel SDF produced strong growth-promoting activity against *B. longum*, *B. infantis*, and *L. brevis*. However, the pulp and peel SDFs showed weak and moderate growth-promoting activities at 5 mg addition groupes against *E. coli* and *C. perfringens*, resulting in strong growth-inhibiting activities to the harmful intestinal microorganisms as compared to glucose. The results of this study indicate that the pulp and peel SDFs could be good substrates for intestinal bacteria and aid in improving the colonic environment.

Many investigations have been performed to find naturally occurring selective growth promoters against beneficial intestinal microorganisms, as well as growth inhibitors against harmful intestinal microorganisms, thus resulting in many candidate materials from medicinal plants (37,38) and DFs (23,24,39) with beneficial health effects on colonic environmental conditions. Choi *et al.* (24) reported on the growth-inhibiting activity of the stem and root bark alcohol insoluble residues from *Ulmus davidiana* against *E. coli*. Also, Jun *et al.* (23) showed that the pectic substance fractions of alcohol insoluble polysaccharides extracted from cucumber peel had different growth-promoting or -inhibiting activities to intestinal bacteria.

In conclusion, although the SDFs extracted from wax gourd pulp and peel had different physichochemical properties such as DF content, monosaccharide composition, molecular weight distribution, and ion exchange chromatographic properties, the pulp SDF had high glucose retardation effects and strong growth-inhibiting activities against *E. coli* and *C. perfringens*, while the peel SDF had strong growth-promoting activity against *B. longum*, *B.*

Table 4. Effects of SDFs from wax grourd pulp and peel on the growth of intestinal bacteria in reinforced clostridial medium broth[1)]

Microoganism	Glucose			Pulp			Peel		
	1 mg	3 mg	5 mg	1 mg	3 mg	5 mg	1 mg	3 mg	5 mg
Bifidobacterium longum	+	+++	++++	+	+	++	+	+	+++
Bifidobacterium infantis	+	+++	++++	+	+	++	+	+	+++
Bifidobacterium bifidum	+	+++	++++	+	+	++	+	+	++
Lactobacillus brevis	+	+	++++	+	+	+	+	+	+++
Escherichia coli	+	+	+++	+	+	+	+	+	++
Clostridium perfringens	+	++	+++	+	+	+	+	+	++

[1)]Judgement of bacterial growth: ++++ (strongest), 2.5>GIR; +++ (strong), 2.0<GIR < 2.4; ++ (moderate), 1.5<GIR<1.9; + (weak), 1.0<GIR< 1.4; - (no response) GIR, GIR<1.0; GIR (growth increase rate)=(A_{600} sample $-A_{600}$ bacteria)/A_{600} control.

infantis, and *L. brevis*, suggesting that wax gourd pulp and peel could be good SDF sources.

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