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Physicochemical Properties and Intestinal Bacterial Growth-Promoting Effect of Cell-Wall Polysaccharides from Cucumber Peel

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Abstract Physicochemical properties, intestinal microbial growth, and inhibitory effects of alcohol-insoluble polysaccharide (AIP) extracted from cucumber peel were investigated. AIP was composed of 14.54% crude protein, 1.04% crude lipid, 13.74 % crude ash, 9.1% soluble dietary fiber, and 41.2% insoluble dietary fiber. AIP showed low bulk density (0.18 g/mL) and water-holding capacity (6.39 g/g), and high oil-holding capacity (3.96 g/g). Pectic substance fractions [water-soluble pectic substance (WSP), ethylenediaminetetraacetic acid-soluble pectic substance (ESP), and alkali-soluble pectic substances (ASP)] and hemicellulose fractions [1 M KOH-soluble hemicellulose (KHP1) and 4 M KOH-soluble hemicellulose (KHP4)] were obtained from sequential chemical fractionation of AIP. WSP showed higher total sugar contents than total uronic acid contents, whereas opposite results were observed in ESP and ASP. Molecular weight distributions of three pectic substance fractions were in order of ASP>ESP>WSP. Ion exchange chromatogram pattern of WSP was different from those of ESP and ASP. Major component of WSP was fraction eluted by 0.05 M ammonium acetate buffer, whereas that of ESP and ASP was fraction eluted by 0.2 M NaOH. WSP and ASP showed growth-promoting activities against *Lactobacillus brevis, Bifidobacterium bifidum*, and *B. longum*, whereas *B. bifidum* and *B. longum* for ESP. KHP1 and KHP4 fractions had significant growth-promoting activities against *B. bifidum*.

Keywords: alcohol-insoluble polysaccharide, cucumber peel, pectic substance fractions, hemicellulose fraction, intestinal bacteria

Introduction

Major fractions of plant cell wall are generally composed of pectins, hemicelluloses, cellulose, proteins, and a very small amount of lignin. These components are alcohol-insoluble polysaccharide (AIP) and cannot be digested by endogenous enzymes secreted from the digestive tract. Of much recent interests are the physiological functions of AIP, such as blood cholesterol- and glucose-lowering effects (1, 2), and gastrointestinal health-promoting effect (3). Both the growth promotion of beneficial intestinal bacteria and the growth inhibition of harmful intestinal bacteria, in particular, would be most important to the maintenance of healthy human gut. In addition, recent studies reported that physiological functions of pectin are related to its physicochemical properties (4, 5). Therefore, interest has been focused on AIPs in non-cereal byproducts or pomace from fruits and vegetables to investigate their physicochemical properties, physiological activities, and potential applications (6-10).

Cucumber (*Cucumis sativus* LINN) is a plant of the Cucurbitaceae family and consumed as a popular summer vegetable. Vitamins and minerals have been recognized as good sources of nutritional values of this vegetable for a long time. In addition, this vegetable possesses various health benefits, such as diuretic action and cleaning action within the body, by removing the accumulated pockets of old waste material and chemical toxins. All-year-round large amounts of cucumber are harvested throughout Korea; however, the cucumber peel as an agricultural by-product is discarded. Although vegetable and fruit peels, rich in pectin, are good dietary fiber sources, they tend to spoil rapidly due to their high moisture content and the presence of various enzymes. Therefore, excess fresh fruits and vegetables are made into powder to improve storage stability and are applied in many types of processed foods.

The objectives of this study were to investigate the physicochemical properties of AIP from cucumber peel and the effects of AIP fractions on human intestinal bacteria *in vitro* to evaluate the feasibility of cucumber peel as a pectin source.

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Materials and Methods

Materials Cucumber was purchased from a local market. Solvents used for the preparation of AIP were obtained from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). Dimethyl sulphoxide (DMSO) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were of analytical grade.

Preparation of AIP Cucumber peel was freeze-dried, ground, shifted using a 100-μm sieve, and stored in a deep freezer (-60°C). AIP was prepared using the method described by Rose *et al.* (11) with a minor modification. Cucumber peel powder (2 g, dry basis) was homogenized in 95% ethanol (200 mL), boiled for 1 hr at 80°C, and filtered through a glass filter (1G4). The residue was washed sequentially with boiling ethanol (200 mL), chloroform:methanol mixture (1:1 v/v, 200 mL), and

acetone (200 mL). After adding 90% DMSO (9:1 v/v, 100 mL) to remove starch, the residue was shaken for 24 hr, filtered through a glass filter (1G4), and air-dried overnight at 40°C.

Sequential fractionation of AIP Sequential fractionation (Fig. 1) of AIP was based on the method described by Murayama *et al.* (12). AIP (1 g) was dispersed in distilled water (25 mL), shaken in a shaking incubator (20°C, 250 rpm) for 24 hr, and centrifuged at 14,000 × g for 20 min. The solution was then filtered through a glass filter (1G4), and the insoluble materials were washed with distilled water (5 mL). Filtrates were combined and freeze-dried [water-soluble pectic substance (WSP)]. The residue (R_1) was homogenized in 0.05 M EDTA-Na solution (ethylenediaminetetraacetic acid-Na, pH 6.5, 25 mL), shaken in a shaking incubator (20°C, 250 rpm) for 24 hr, and centrifuged at 14,000 × g for 20 min. The solution was then filtered through a glass filter (1G4), and the insoluble materials were washed with 0.05 M EDTA-Na solution (5 mL). Filtrates were combined and freeze-dried [EDTA-soluble pectic substance (ESP)]. The residue (R_2) was homogenized in 0.05 M Na_2CO_3 solution (25 mL) containing 0.02 M $NaBH_4$ solution, shaken in an incubator (20°C, 250 rpm) for 24 hr, and centrifuged at 14,000 × g for 20 min. The solution was then filtered through a glass filter (1G4), and the insoluble materials were washed with 0.05 M Na_2CO_3 solution (5 mL) containing 0.02 M $NaBH_4$. The filtrates were combined, neutralized with acetic acid, and freeze-dried [alkali-soluble pectic substances (ASP)]. The residue (R_3) was homogenized in 1 M KOH (25 mL) containing 0.02 M NaBH, shaken in an incubator (20°C, 250 rpm) for 24 hr, and centrifuged at 14,000 × g for 20 min. The solution was then filtered through a glass filter (1G4), and the insoluble materials were washed with 1 M KOH. The filtrates were combined, neutralized with acetic acid, and freeze-dried [1 M KOH-soluble hemicellulose (KHP1)]. The residue (R_4) was homogenized in 4 M KOH (25 mL) containing 0.02 M $NaBH_4$ solution, shaken in an incubator (20°C, 250 rpm) for 24 hr, and centrifuged at 14,000 × g for 20 min. The solution was then filtered through a glass filter (1G4), and the insoluble materials were washed with 4 M KOH. The filtrates were combined, neutralized with acetic acid, and freeze-dried [4 M KOH-soluble hemicellulose (KHP4)]. The residue (R_5) was freeze-dried after washing with 95% ethanol and acetone (cellulose).

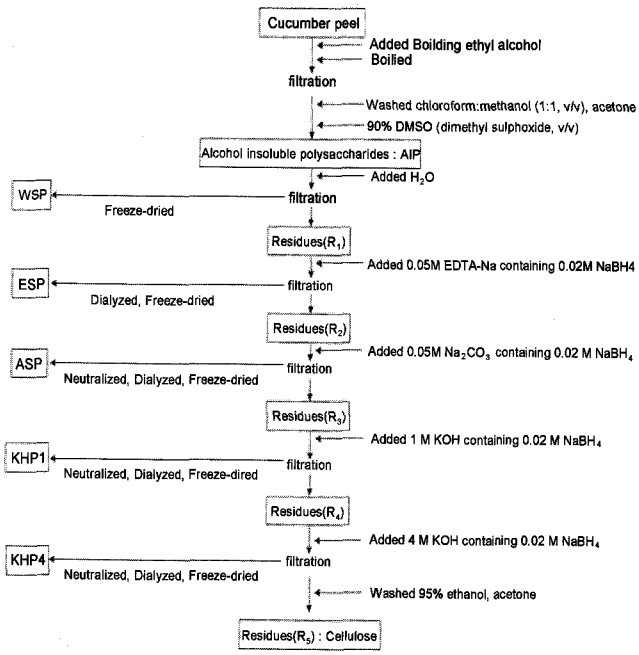

Fig. 1. Scheme for isolation of alcohol insoluble polysaccharide fractions from cucumber peel.

Chemical analysis Crude protein, crude lipid, and crude ash contents were estimated by the AOAC official methods (13). Dietary fiber content was determined using dietary fiber assay kit (Sigma Chemical Co.) after enzymatic removal of starch and protein (14). Samples (1 g) were dispersed in phosphate buffer (0.08 M, pH 6.0 ± 0.2) to analyze the soluble dietary fiber (SDF) and insoluble dietary fiber (IDF).

Physical properties To measure the bulk density, the sample was filled into a graduated cylinder, which was then tapped gently against the table top, and weighed (3). Results were expressed as weights of samples per cylinder bulk of samples. The water-holding capacity (WHC) was measured by centrifugation (15). One gram of sample was placed in 50 mL centrifuge tube, to which 25 mL distilled water was added. The mixture was mixed in a Vortex mixer at high speed for 1 min, maintained for 1 hr at room temperature, and centrifuged at 12,000 × g for 30 min. The supernatant was removed carefully, and the tube was inverted for 15 min on the filter paper to remove distilled water. Moisture content of the precipitate was determined by drying at 105°C overnight in a forced-air oven. The oil-holding capacity (OHC) was determined by adding commercial soybean oil (25 mL) to the sample (1 g) in 50 mL centrifuge tube (3). The samples were mixed in a Vortex mixer at high speed for 1 min and left for 1 hr at room temperature before centrifugation for 30 min at 12,000 × g. The supernatant was removed carefully, and the tube was inverted for 15 min on the filter paper to remove oil. The residue was weighed, dried for 24 hr at 60°C in a forced-air oven, and weighed again. Results were expressed as g of soybean oil per g of dry sample.

Analysis of total sugar and total uronic acid Total sugar and total uronic acid contents of the extracted fractions were determined by phenol-sulfuric acid method (16) and *m*-hydroxydiphenyl method (17), respectively.

Gel filtration and ion exchange chromatography Gel filtration chromatography was performed on a Pharmacia column (2.6 × 100 cm) of Sepharose CL-6B by elution with 0.1 M Na-phosphate buffer (pH 6.0) at 0.5 mL/min. Dextran 2,000 kDa, dextran 513 kDa, dextran 43 kDa, and dextran 10 kDa (Sigma Chemical Co.) were used as molecular weight markers. Samples (300 mg) were solubilized in 6 mL of 0.1 M Na-phosphate buffer (pH 6.0), and supernatants (2 mL) from the aqueous layer were applied on the column. Fractions (10 mL) were collected to analyze total sugar and total uronic acid contents.

Ion exchange chromatography was performed on a Pharmacia column (2.6 × 30 cm) of DEAE Sepharose CL-6B. The column was eluted with 0.05 M ammonium acetate buffer, followed by 0.1 - 1 M linear gradient of ammonium acetate buffer (pH 6.0), and finally 0.2 M NaOH (fraction 76-90) at 1 mL/min. Samples (300 mg) were solubilized in 6 mL distilled water, and the supernatants (2 mL) from the aqueous layers were applied on the column. Fractions (10 mL) were collected to analyze the total sugar and total uronic acid contents.

Effect of fractionated polysaccharides on the growth of intestinal bacteria Five intestinal bacterial strains (*Escherichia coli* KCTC 2441, *Clostridium perfringens* KCTC 3269, *Lactobacillus brevis* KCTC 3102, *Bifidobacterium bifidum* KCTC 3202, and *B. longum* KCTC 3128) activated in BHI and MRS broth media were used. The bacterial culture was anaerobically cultivated with AnaeroGem™ pack (Oxoid Ltd., Basingstoke, England) and Anaero Jar (Oxoid Ltd.) at 37°C for 48 hr. The growth of microorganisms was measured using the method of Lee and Kim (18) with a slight modification. The experimental group was composed of 4.7 mL RCM, 100 μL bacteria, and 200 μL sample. The bacteria control group was composed of 4.7 mL RCM, 100 μL bacteria, and 200 μL distilled water, while the sample control group was composed of 4.7 mL RCM, 100 μL distilled water, and 200 μL sample. Samples were autoclaved after dissolving 5 mg each of AIP fraction per 200 μL distilled water. The bacterial growth was measured spectrophotometically at 600 nm. Growth-promoting response was expressed as growth increase rate (GIR = A_{600} sample+bacteria - A_{600} sample control / A_{600} bacteria control).

Statistical analysis Data were analyzed by ANOVA using the SAS statistical analysis system (SAS Institute Inc., Cary, NC, USA) (19). Differences among the samples were analyzed using the Duncan's multiple range test at a 5% significance level.

Results and Discussion

Physicochemical properties of AIP The yield and proximate composition of cucumber peel AIP are given in Table 1. The content of AIP was 72.48±0.06%, which was much higher than those previously reported for fruit byproducts, and was composed of 14.54±0.14% crude protein, 1.04±0.16% crude lipid, and 13.74±0.10% crude ash (6, 20). AIP also contained 9.1±0.05% soluble dietary fiber, 41.2±0.16% insoluble dietary fiber, and 50.3±0.11% total dietary fiber, indicating it as a good dietary fiber source. The results obtained in present study were much lower than those obtained from apple and artichoke dietary fiber concentrates, but higher than those found by Grigelmo-Miguel and Martin-Belloso (21) in pear, orange, peach, asparagus dietary fiber concentrates as well as cereal brans.

The bulk density, WHC, and OHC of AIP from cucumber peel are shown in Table 1. The bulk density, which could be affected by the particle geometric forms, porosity, moisture content, particle distribution, and cell wall structure, of cucumber peel AIP was 0.18 g/mL,

Table 1. Proximate composition and physical properties of AIP from cucumber peel

Proximate composition	AIP content (%)	72.48±0.06[1)]
	Crude protein (%)	14.54±0.14
	Crude lipid (%)	1.04±0.16
	Crude ash (%)	13.74±0.10
	Soluble dietary fiber (%)	9.10±0.05
	Insoluble dietary fiber (%)	41.20±0.16
	Total dietary fiber (%)	50.30±0.21
Physical properties	Bulk density (g/mL)	0.18±0.00
	WHC (g/g)[2)]	6.39±0.05
	OHC (g/g)[3)]	3.96±0.06

[1)]Determined in triplicate (mean±SD).
[2)]WHC = water holding capacity.
[3)]OHC = oil holding capacity.

much lower than those previously reported for dietary fibers from agricultural byproducts (6, 7, 22). WHC of cucumber peel AIP was 6.39±0.05 g/g. This value was relatively lower than those previously reported for dietary fiber concentrates from various fruit byproducts, which could be attributed to the low amount of soluble fractions, as well as to the physical properties such as particle size, porosity, and physical structure of the fiber matrix (7, 21, 23, 24). OHC of cucumber peel AIP was 3.96 ± 0.06 g/g, much higher than those previously reported for dietary fibers from agricultural byproducts (7, 8, 23).

Fractionation of AIP and sugar composition AIP is generally composed of pectic substances, hemicelluloses, cellulose, and small amount of glycoproteins (11). Therefore, to obtain detailed information on the components of AIP, AIP is generally fractionated through treatment with weak acid or alkali, followed by strong alkali (25).

Three pectic substance fractions (WSP, ESP, and ASP) and hemicellulose fractions (KHP1 and KHP4) were obtained from sequential chemical fractionation of AIP extracted from cucumber peel (Table 2). Total sugar contents were higher than total uronic acid contents in WSP, whereas the opposite trend was observed in ESP and ASP. Uronic acid content was much higher in ESP and ASP fractions than in WSP fraction, about 300 mg/g in

Table 2. Total sugar and total uronic acid contents of AIP fractions from cucumber peel

Fractions[1)]	Total sugar (mg/g)	Total uronic acid (mg/g)
WSP	174.66±2.38[2)]d[3)]A[4)]	134.77±2.21bB
ESP	222.71±1.33cB	303.36±8.43aA
ASP	259.84±3.82bB	302.86±5.71aA
KHP1	272.85±3.54aA	55.40±3.05cB
KHP4	131.74±0.69eA	20.13±2.75dB
Cellulose	32.57±0.89fA	26.53±1.96dB

[1)]WSP: water soluble pectic substances, ESP: EDTA soluble pectic substances, ASP: alkali soluble pectic substances, KHP1: 1 M KOH solulbe hemicelllulose, KHP4: 4 M KOH soluble hemicellulose.
[2)]Determined in triplicate dry samples(mean±SD).
[3)]Followed by different letters within columns are significantly different at p=0.05 by duncan's multiple test.
[4)]Followed by different letters within row are significantly different at p =0.05 by duncan's multiple test.

both ESP and ASP. This value was over 5.5 times higher than those of hemicellulosic and cellulosic fractions, possibly because uronic acid was the main component of pectic substance. Among these pectic substance fractions, the uronic acid content of WSP was lower than those of ESP and ASP, possibly because WSP faction is typically thought to include polymeric material that has been solubilized from cell wall, whereas the ESP and ASP fractions are generally considered to be enriched for ionically and covalently bound pectins, respectively (11).

Gel and ion exchange chromatographies of AIP fractions To compare the molecular weight distributions of AIP fractions, each fraction was subjected to gel filtration chromatography on Sepharose CL-6B (Fig. 2). The molecular weight distributions of three pectic substance fractions differed from each other. WSP gave two peaks of total sugar with average molecular masses close to 250 and 16 kDa on the basis of calibration with dextrans. ESP had a broad population of polymers peaking between 2,000-519 kDa, whereas the molecular weight distribution of ASP showed a tailing peak with top near 2,000 kDa. The order of molecular distributions was ASP>ESP>WSP. Uronic acid peaks were also detected at approximately 13 and 250 kDa for WSP, 51, 205, and 1,480 kDa for ESP, and 138, 371, 818, and 1,804 kDa for ASP. It should be noted, however, that polysaccharides containing uronic acids, due to intramolecular electrostatic repulsions by charge effects, have a different hydrodynamic volume than dextrans and, therefore, are eluted at different rates than expected on the basis of their molecular weight (26). In the case of hemicellulosic fractions, three total sugar peaks close to 35, 304, and 1804 kDa, and 51, 371, and 2,198 kDa were identified for KHP1 and KHP4, respectively. The uronic acid peaks were also identified at 35, 205, and 1,804 kDa, and 35, 138, and 1,804 kDa for KHP1 and KHP4, respectively; however, the ratio of total sugar to uronic acid decreased significantly.

To obtain more information on the molecular properties of AIP, cucumber peel AIP fractions were subjected to anion exchange chromatography on DEAE sepharose CL-6B using a buffer gradient to afford several fractions (Fig. 3). In the case of pectic substance fractions, the peak pattern of WSP was different from those of ESP and ASP. Fraction F1 of WSP, which was eluted by 0.05 M ammonium acetate buffer, was the major component of WSP, and the sugar content of fraction F1 was much higher than uronic acid content. The finding that fraction

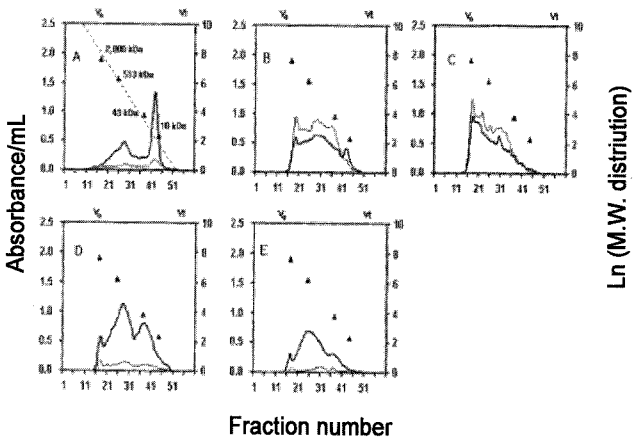

Fig. 2. Gel filtration profiles of pectic substance fractions from cucumber peel on Sepharose CL-6B. A: WSP fraction; B: ESP fraction; C: ASP fraction, D: KHP1 fraction, E: KHP4 fraction. Column fractions were assayed for uronic acid (light line) and for total sugar (heavy line).

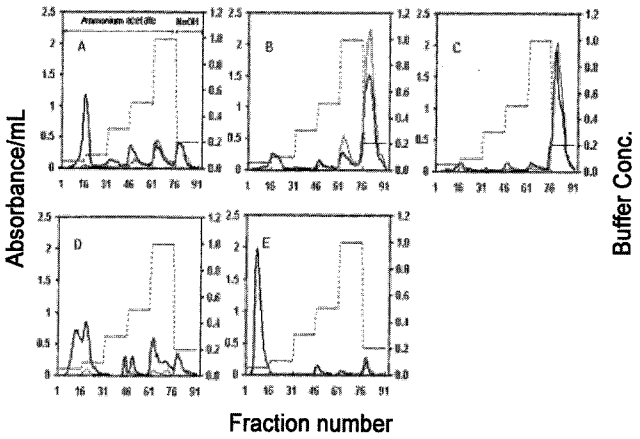

Fig. 3. Ion exchange profiles of pectic substance fractions from cucumber peel on DEAE Sepharose CL-6B. A: WSP fraction; B: ESP fraction; C: ASP fraction, D: KHP1 fraction, E: KHP4 fraction. Column fractions were assayed for uronic acid (light line) and for total sugar (heavy line).

Table 3. Effect of AIP fractions from cucumber peel on the growth of intestinal bacteria in RCM broth

Fractions[1)]	GRI[2)]				
	Escherichia coli	*Clostridium perfringens*	*Lactobacillus brevis*	*Bifidobacterium bifidum*	*Bifidobacterium longum*
WSP	1.04±0.02[3)]a[4)]C[5)]	1.25±0.06aC	1.11±0.03cC	2.20±0.04aA	1.67±0.28bB
ESP	0.48±0.01cD	0.02±0.00dE	1.10±0.03cA	0.55±0.03cC	0.64±0.02cB
ASP	0.93±0.03bB	0.14±0.04cC	1.80±0.07aA	1.99±0.14bA	1.97±0.17aA
KHP1	0.99±0.03aC	0.94±0.07bC	1.93±0.20aB	2.23±0.11aA	1.87±0.04abB
KHP4	0.93±0.02bD	0.99±0.08bD	1.45±0.08bC	2.22±0.10aA	1.79±0.08abB

[1)]WSP: water soluble pectic substances, ESP: EDTA soluble pectic substances, ASP: alkali soluble pectic substances, KHP1: 1 M KOH soluble hemicelllulose, KHP4: 4 M KOH soluble hemicellulose.
[2)]GRI(growth increase rate): (A_{600} sample-A_{600} bacteria) / A_{600} control.
[3)]Determined in triplicate dry samples(mean±SD).
[4)]Followed by different letters within columns are significantly different at p=0.05 by duncan's multiple test.
[5)]Followed by different letters within row are significantly different at p=0.05 by duncan's multiple test.

F1 of WSP could be eluted under low ionic strength condition may be related to the lower content of uronic acid. The fractions which had higher contents of uronic acids, F3, F4, and F5, also appeared from elution using higher molar concentration of buffer. The patterns of ion exchange chromatograms of ESP and ASP were similar, and elution with 0.2 M NaOH afforded a major peak (fraction F4). The uronic acid contents of fraction F4 in ESP and ASP were higher than the sugar contents. It is generally accepted that ESP and ASP fractions are enriched for ionically and covalently bound pectins (11). In the case of hemicellulosic fractions, KHP1 had very low uronic acid contents. Uronic acid peaks were identified at 0.05, 0.1, and 1 M ammonium acetate and 0.2 M NaOH, while KHP4 was identified at 0.05 M ammonium acetate and 0.2 M NaOH.

Effect of fractionated polysaccharides on the growth of intestinal bacteria The growth-promoting and -inhibiting activities of AIP fractions from cucumber peel on human intestinal bacteria are shown in Table 3. WSP and ASP showed growth-promoting activities against *L. brevis, B. bifidum*, and *B. longum*, which are good intestinal microorganisms, whereas ESP showed growth-inhibiting activity against *L. brevis*. WSP also showed slight growth-promoting activity against *E. coli* and *C. perfringens*, which are harmful intestinal microorganisms. ESP and ASP inhibited the growths of *E. coli* and *C. perfringens*, with ESP, in particular, showing a significant inhibition against the growth of *C. perfringens*. For hemicellulose fractions, KHP1 and KHP4 had significant growth-promoting activities against *B. bifidum*. These results indicated that WSP, ASP, KHP1, and KHP4 fractions from cucumber peel could improve the gastro-intestinal health. The strong growth-promoting effects of these AIP fractions on *Bifidobacterium* and *Lactobacillus* could also contribute to the prevention of diseases caused by pathogens in human gut.

Acknowledgments

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