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ARTICLE

Soluble Polysaccharide Composition and *myo*-Inositol Content Help Differentiate the Antioxidative and Hypolipidemic Capacity of Peeled Apples

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Many people prefer to eat peeled apples. The present study investigated the composition of soluble polysaccharides (SP) in peeled apples and its antioxidative and hypolipidemic activity. The yield of SP ranged 0.43−0.88%, having MW ranging 223−848 kDa. All belonged to peptidoglycans. Among the fourteen amino acids found, seven were essential amino acids. In addition, sugar analysis indicated that 50% of apple samples consisted of glucoarabinan, 37.5% comprising taloarabinan and the remaining 12.5% containing alloglucan. Moreover, SP consisted of a huge amount of *myo*-inositol (>5.61%) and uronic acid (>11.7%), which may play a synergistic role in the hypolipidemic effect. Worth noting, we are the first who reported the presence of talose, allose and fucose in the apple SP. Conclusively, the biological value of SP is attributable to the differential effect of SP and the synergistic effect exerted by its unique SP pattern, high *myo*-inositol and uronic acid contents.

KEYWORDS: Soluble polysaccharide; peptidoglycan; *myo*-inositol; hypolipidemic; antioxidative

INTRODUCTION

Total dietary fiber of apple, carrot, cauliflower, cabbage, pea, plum, and guava is reported to range 14.68−78.21% on the dry basis (*1*). Upon enzymatic hydrolysis, apple pomace undergoes saccharification and releases mono- and oligosacccharides and some polysaccharides (SP) in its water-soluble fraction (*2*).

Dietary fibers provide greater inhibition of starch digestion, glucose absorption, and glycemic response following starch loading. Most fiber sources are able to decrease the fasting serum level of cholesterol and triglycerides. Simultaneously, the fecal transit time is shortened, the fecal values are raised, and the β-glucuronidase activity in intestinal segment contents and feces is lowered (*3*). High-fiber diets are beneficial for prevention of constipation and the lowering the risk of digestive conditions, reducing blood cholesterol levels, controlling blood sugar levels, aiding in weight loss, and minimizing the risk of colorectal cancer (*4*). Certain beneficial effects of dietary fibers may be mediated by short-chain fatty acids produced during anaerobic fermentation in the colon (*5*). Consumption of viscous indigestible SP may change the exocrine pancreatic biliary function and depress the process of digestion and absorption, and as compensation, a hyperplasia/hypertrophy of digestive organs and increased secretion of digestive juice could be assessed (*6*). Moreover, SP can readily be fermented and degraded by the intestinal microflora to produce smaller oligosaccharides acting as immune enhancers (*7*), thus underlying the postdigestion effect of dietary fibers on physiological and biochemical changes. In Western society, apples are usually considered to be the best nutraceutic fruits in the prevention of chronic diseases. This study examined the composition of SP of commonly consumed apple cultivars in the market and compared their antioxidative and hypolipidemic bioactivities.

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MATERIALS AND METHODS

Origin and Species of Apples. Eight species of apples were collected from the local market, thirty for each. These cultivars involved *Malus domestica* "Fuji" (Japan origin, designated sample A1), weighing within 100.7 ± 5.8 g; *Malus domestica* "Jonagold" (Korea origin, designated A2), with weight range 100.1 ± 6.2 g; *Malus domestica* "Golden Delicious" (Japan origin, designated A3), weighing within 104.0 ± 6.8 g; *Malus domestica* "Gala" (Japan origin, designated A4), weight ranging within 100.0 ± 4.7 g; *Malus domestica* "Arkansas Black" (Japan origin, designated A5), having weight range 101.2 ± 5.6 g; *Malus domestica* "Fuji" (USA origin, designated A6), weighing within 103.4 ± 6.4 g; *Malus domestica* "Granny Smith" (USA origin, designated A7), weight ranging within 100.0 ± 6.6 g; and *Malus domestica* "Red Delicious" (USA origin, designated A8), having weight range 101.0 ± 6.2 g, respectively.

Preparation of SP. According to the method of AOAC (1995) (*8*) and following the instruction given by the manufacturer, the determination of dietary fibers was carried out using the Fiber Assay Kit (Sigma-Aldrich, St. Louis, MO). Briefly, apples were peeled with a stainless peeling knife. One gram of peeled sample was ground and passed through a mesh #60 stainless sieve. To the pulp 40 mL of 0.05 M Mes-Tris buffer (pH 8.2) and 50 μL of thermostable α-amylase (EC 3.2.1.1, A3306, Sigma-Aldrich, St. Louis, MO) were added. The reaction mixture was heated at 95−100 °C

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Table 1. Diet Formula for Animal Experiment (g/100g)[a]

ingredients	C	H	A1	A2	A3	A4	A5	A6	A7	A8
casein	20	20	20	20	20	20	20	20	20	20
sucrose	15	15	15	15	15	15	15	15	15	15
corn starch	50	45	45	45	45	45	45	45	45	45
corn oil	2.5	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
lard	2.5	5	5	5	5	5	5	5	5	5
cholesterol		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
M_P[b]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
V_P[c]	1	1	1	1	1	1	1	1	1	1
choline	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
α-cellulose	5	5.0	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
A1SP			0.5							
A2SP				0.5						
A3SP					0.5					
A4SP						0.5				
A5SP							0.5			
A6SP								0.5		
A7SP									0.5	
A8SP										0.5

[a] Data of ingredients formulated were in absolute weight, g. C: control diet. H: high fat high cholesterol diet. The following diets (A1−A8) contained 0.5 g of relevant sample soluble polysaccharides (SP) formulated with diet H. A1: *Malus domestica* "Fuji" (Japan origin). A2: *Malus domestica* "Jonagold" (Korea origin). A3: *Malus domestica* "Golden Delicious" (Japan origin). A4: *Malus domestica* "Gala" (Japan origin). A5: *Malus domestica* "Arkansas Black" (Japan origin). A6: *Malus domestica* "Fuji" (USA origin). A7: *Malus domestica* "Granny Smith" (USA origin). and A8: *Malus domestica* "Red Delicious" (USA origin). [b] Mineral premix. [c] Vitamin premix.

in a water bath for 30 min. When cooled to 60 °C, 100 μL of threonine endopeptidases (Protease EC3.4.25, Sigma-Aldrich, St. Louis, MO) and 5 mL of 0.561 N HCl were added. The heating was continued with gentle shaking at 60 °C for 30 min. The pH value was adjusted to 4.0 with either 1 N HCl or 1 N NaOH. To the mixture 300 μL of amyloglucosidase (EC 3.2.1.3. *Aspergillus niger*, Type A3513, Sigma-Aldrich, St. Louis, MO) was added, and the mixture was heated at 60 °C in a water bath for 30 min to facilitate the hydrolysis. The hydrolyzed solution was filtered (HS). To HS a 4-fold volume of ethanol (95%) was added and left to stand at 24 °C for 1 h to initiate precipitation. The precipitate was collected on a Whatman No. 2 filter paper and successively rinsed once with 40 mL of ethanol (95%) and twice with 40 mL of acetone, respectively. The residue was oven-dried at 105 °C overnight. The weight of the desiccated precipitate was taken. The percent yield of SP was calculated with correction for its crude protein and ash contents. The SP obtained was incorporated into the experimental diet formulation (**Table 1**). Similarly, a blank test was performed with similar procedures using all reagents exclusive of the test sample.

Carbohydrate Content in SP. The carbohydrate content was determined by the Dubois phenol−H_2SO_4 colorimetric method (*9*), which is applicable only for the determination of neutral sugars like glucose, galactose, mannose, etc. Briefly, the SP was accurately weighed and dissolved in double distilled water (ddw) to obtain the stock solution of SP (10 mg/mL). The stock solution was diluted with ddw to make concentrations of 0.1, 0.2. 0.3, 0.4, 0.5, and 0.6 mg/mL, respectively. To each 0.5 mL of SP soluion, 0.5 mL of phenol color reagent (5%) was added. The mixture was vigorously agitated to facilitate a homogeneous solution. To the solution 2.5 mL of sulfuric acid was rapidly dropped in, and the mixture was agitated thoroughly to facilitate the color reaction (orange color). The optical density was measured at 490 nm. The carbohydrate content was calculated and expressed in % w/w. A corresponding concentration of authentic glucose solution was similarly treated and used as the reference standard.

Molecular Weight Determination by Gel Permeation Chromatography (*GPC*). Sample SP (10 mg) was accurately weighed and transferred into the reaction vessel, to which 1 mL of 1 N NaOH was added. The mixture was heated at 60 °C in a water bath until dissolved. To the solution 2 mL of deionized water was added, and the solution was agitated for 10 min and then filtered through a 0.45 μm micropore to obtain the alkali dissolvable polysaccharides (ADP). For determination of the molecular weight, GPC was conducted according to Ker et al. (*8*). Briefly, to each 0.5 mL of ADP 1 mL of NaOH and sufficient double distilled water were added to obtain a final volume of 5 mL. The mixture was thoroughly agitated to facilitate the dissolution. The solution was centrifuged at 2500 rpm for 10 min to precipitate the insoluble suspensions. The supernatant was decanted. Three milliliters of each was eluted on the Sephadex G-100 column (2.5 × 100 cm) with 0.05 N NaOH solution containing 0.02% of NaN_3 at a flow rate 0.5 mL/min. The eluent was collected by a fraction collector (ISCO Retriever 500, Isco., Lincoln, NE), 6 mL in each. A total of fifty tubes were collected. The optical density was simultaneously scanned at 490 and 280 nm, respectively. The molecular mass distribution and mean molecular mass were calibrated by the standard curve established by authentic dextrans (Sigma, St. Louis, MO) having known molecular masses (8.8, 40, 500, 2000, and 5000−40000 kDa, respectively). The average molecular mass was calculated by linear correlation between the logarithm of the molecular mass of the standards and the ratio of their elution volumes to the void volume of the column (*9*).

Table 2. Retention Time of Different Monosaccharides

standard/sample	retention time (min) (range[a])
D-deoxyribose	19.669 (19.06−19.916)
L-rhamnose	20.459
fucose	20.822 (20.32−21.159)
D-(−)-ribose	21.109
D-lyxose	21.134 (20.682−21.307)
D-(−)-arabinose	21.332
D-(+)-xylose	22.138 (21.76−22.98)
allose	24.196 (23.669−24.377)
talose	24.821 (24.484−25.052)
D-mannose	25.471 (24.838−25.521)
D-fructose(mannose)	25.471
D-galactose	25.932 (25.46−25.998)
D-fructose(glucose)	26.623
D-(+)-glucose	26.623 (25.998−26.887)
glucosamine	27.257
myo-inositol	27.373, 27.529; (27.003−27.685)
lactose	35.108−36.971
sucrose	35.487−37.263

[a] The range is shown in parentheses.

The Monosaccharide Composition in SP: Hydrolysis, Reduction, and Derivatization of Monosaccharides. The method of Blakeney et al. (1983) with slight modification by Ker et al. (*8*) was used. In principle, the purified SP was subjected to complete acid hydrolysis. The hydrolyzed mixture of monosaccharides was acetylated to form acetylated monosaccharides and analyzed with GC/MS. The quantity in mol % of each individual monosaccharide was calculated from the corresponding percent peak area by comparing with the blank. Practically, Ker et al. (*9*) was followed. Two milligrams of SP was accurately weighed and transferred into the Cole-Parmer reactor, to which 2 mL of 2 M trifluoroacetic acid was added. The mixture was heated at 120 °C with the Cole-Parmer heater for 24 h while shaken vigorously every 30 min during heating until completely hydrolyzed. The hydrolyzed mixture was subjected to nitrogen blow until dried. The residue was dissolved twice with NH_4OH solution. Each time 100 μL of 1 M NH_4OH containing 1 mg/mL of deoxyribose was used as the internal standard. (Should there be any mono- or disaccharide initially existing in the sample, 5 mg of this should be accurately weighed and dissolved with 200 μL of 1 M NH_4OH.) The reduction was conducted in the Cole-Parmer reactor with 0.2 g of $NaBH_4$/10 mL of DMSO. The remaining procedures were conducted following Ker et al. (*9*). The final deduced product was transferred into a 1 mL reaction vessel, lyophilized and analyzed with GC/MS as described (*9*).

GC/MS Analysis. GC/MS was conducted in the Restek Rtx 225 column (l × i.d. = 30 m × 0.32 mm). The temperature was programmed, initially at 60 °C for 1 min, raised at an elevation rate 8 °C/min until 220 °C, and held this temperature for 10 min. The temperatures at the injection port and the detector were set equally at 230 °C. Hydrogen was used as the carrier gas and operated at a flow rate of 2.0 mL/min. The reference values of retention time of the standard monosaccharides are shown in **Table 2**.

Analysis For Proteins. The method of Bradford was followed to determine the protein content (*10*). Briefly, 5.1 mg of SP was accurately weighed, to which a tiny amount of NaOH (1 N) was added. The mixture was agitated to facilitate the dissolution. The solution was made to a volume of 5 mL with 1 N NaOH. Ten microliters of each standard and/or sample solution was transferred into a 96-well microplate. To each well 200 μL of diluted dye reagent was added. The mixture was gently agitated and incubated at ambient temperature for 5 min. The optical density was measured at 595 nm with an ELISA reader. A calibration curve was established using authentic bovine serum albumin (BSA), from which the amount of protein present was calculated and expressed in percent w/w.

Acid Hydrolysis. Sample SP (3 g) was accurately weighed and transferred into a 2 mL reaction vessel, to which 2 mL of 6 M HCl was added. The mixture was subjected to nitrogen blowing for 10 min to drive off the dissolved oxygen. The reaction vessel was sealed and heated at 110 °C for 24 h until completely hydrolyzed. The product amino acid mixture was lyophilized. The desiccated product was dissolved in 0.3 mL of 0.01 M HCl to obtain the sample of hydrolyzed amino acid mixture (AAM).

Derivatization of Amino Acid and Extraction. 0.3 mL of authentic sample solution and 0.6 mL of AAM were respectively placed into a 3 mL reaction vessel, to which 0.01 mL of internal standard norleucine solution (10 mg/mL) was added. The mixture was vigorously agitated, and 0.1 mL of ethyl chloroformate was added. After the mixture was well shaken, 1 mL of alcohol−pyridine was added. On addition of 2 mL of chloroform, the mixture was vigorously agitated for 1 min to facilitate derivatization. To the reaction mixture 0.7 mL of water was added. The mixture was shaken well and left to stand for 5 min to facilitate phase separation. The supernatant was discarded. The lower layer (i.e., the chloroform layer) was transferred into another tube. A sufficient amount of anhydrous sodium sulfate was added to enable the dehydration to proceed. The dehydrated chloroform extract was transferred into the sample vessel for GC/MS analysis.

GC/MS Analysis. The GC/MS gas chromatography (Agilent 6890, Wilmington, DE) installed with an FID detector and a column HP-5MS (l = 30 m, i.d. = 0.25 mm, film thickness = 0.25 μm) was used for GC/MS analysis. Nitrogen was used as the mobile phase and operated at a flow rate 0.8 mL/min. The temperature at FID and the injection port was operated at 305 and 300 °C, respectively. The elution process was temperature programmed, initially at 50 °C for 1 min, then at an elevation rate 10 °C/min to 300 °C and held at this temperature for 6.5 min (*9*).

Determination of Uronic Acid. The content of uronic acid was determined with the carbazole−H_2SO_4 method of Cesaretti et al. (*11*). The content of uronic acid was expressed in % w/w.

Hamsters. Eighty-eight male Syrian hamsters, aged 4−5 weeks, were purchased from the National Laboratory Animal Centre. All studies performed with the hamster model were approved by the Hungkuang University Supervising Ethic Committee in accordance with Helsinki Declaration of 1975. The animal room was conditioned at 24 ± 1 °C with a relative humidity maintained at 40−60%. The light cycle was changed every 12 h. Water and meals were taken ad libitum. For the first two weeks, hamsters were acclimated by supplying only regular diets for experimental hamsters (Fu-Sow brand; meaning Long-Live brand in Chinese). The hamsters were then randomly grouped into eleven groups, 8 in each stainless cage. Based on the formula of AIN-76 (1977) (*12*), the experimental diets were prepared (**Table 1**). Each diet was thoroughly mixed to ensure a homogeneous compositional distribution and stored at 4 °C. These hamster groups were fed respectively with diets shown in **Table 1**.

The experimental period extended for 8 weeks, during which the body weight and the amount of diet consumed were recorded every 2 days until the end of the experiment. After 8 weeks, the hamsters were fasted for 12 h and CO_2-anesthetized to collect the blood from the abdominal aorta. Blood was immediately centrifuged at 3200 rpm for 15 min using a KUBOTA-3740 centrifuge to separate and collect the sera, which were used for the determination of total triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). When euthanized, livers were dissected and rinsed twice with saline. The excess adhered water was sucked off the liver surface with tissues, and the net weight of liver was taken.

Analysis of Serum Lipid and Lipoproteins. Serum lipoproteins including TG, TC, LDL-C and HDL-C were determined by the same method previously described by Lin et al. (*13*).

Table 3. The Yield Percent, Mean Molecular Weight, and Contents of Uronic Acid, Carbohydrate, and Protein in the Soluble Polysaccharide Fraction Obtained from Different Species of Apples[a]

sample	yield (%)	MW[b] (kDa)	carbohydrate[c] (%)	protein[d] (%)	uronic acid[e] (%)
A1	0.69	848 ± 88	57.3 ± 1.8	17.5 ± 0.8	24.6 ± 0.6
A2	0.43	631 ± 73	67.2 ± 1.5	9.4 ± 0.4	23.0 ± 0.4
A3	0.77	280 ± 63	47.8 ± 1.6	12.2 ± 0.7	11.7 ± 0.3
A4	0.53	223 ± 53	68.3 ± 1.3	9.8 ± 0.4	22.3 ± 0.4
A5	0.74	244 ± 55	52.4 ± 1.4	10.3 ± 0.5	12.6 ± 0.4
A6	0.71	265 ± 47	58.3 ± 1.7	7.5 ± 0.4	19.2 ± 0.3
A7	0.88	343 ± 56	55.5 ± 1.8	14.0 ± 0.8	15.7 ± 0.3
A8	0.61	652 ± 76	56.2 ± 1.5	10.4 ± 0.6	19.1 ± 0.5

[a] Data expressed as mean ± SD from triplicate determinations. [b] Mean molecular weight (kDa) of soluble polysaccharides obtained from different apple species when determined by GPC. [c] Carbohydrate (%, w/w) was determined by the phenol−H_2SO_4 method. [d] Protein (%, w/w) was determined by BCA protein assay (Bradford, 1976). [e] Uronic acid (%, w/w) was measured by the carbazole−H_2SO_4 method (Cesaretti et al., 2003).

Assay for Glutathione Peroxidase (GPX) and Superoxide Dismutase (SOD).

Glutathione Peroxidase (*GPX*). The method of Mantha was followed (*14*). Briefly, hepatic extract (10 μL) was added with 0.75 mL of reaction solution (2.5 mM GSH, 0.5 mM NaN_3, 0.3 mM EDTA, 0.1 mM NADPH, 0.5 U of glutathione reductase, 0.4 mM *tert*-butyl hydroperoxidase in 50 mM phosphate buffer, pH 7.2). The reaction mixture was left to stand in ambient temperature for 5 min and then combined with 50 μL of 3.125 mM H_2O_2. The mixture was thoroughly mixed. The absorbance was taken at 340 nm against a blank of deionized water. For calibration of enzyme activity, a molar extinction coefficient $\varepsilon_{340} = 6220\ M^{-1}\ cm^{-1}$ was used. One unit of GPX will cause the formation of 1 μmol of $NADP^+$ from NADPH per min at pH 8.0 at 25 °C .

Superoxide Dismutase (*SOD*). According to Marklund (*15*), 10 μL of the hepatic extract was combined with 965 μL of TrisHCl (100 mM) (containing 2 mM of diethylenetriaminepentaacetic acid, pH 8.2) and 25 μL of pryogallol (8 mM in 3 mM HCl). The absorbance was red at 412 nm using deionized water as the blank. The absorbance was red successively within the first 3 min. One unit of SOD is defined as the capacity of 50% inhibition on the auto-oxidation rate of pyrogallol.

Statistics. Data obtained in the same group were analyzed by ANOVA Student's t test with computer statistical software SPSS 10.0 (SPSS, Chicago, IL). Statistical Analysis System (2000) software was used to analyze the variances, and Duncan's multiple range tests were used to test their significances of difference between paired means. Significance of difference was judged by a confidence level of $p < 0.05$.

RESULTS AND DISCUSSION

The total SP obtained from different apple cultivars ranged within 0.43−0.88%. Their molecular weight ranged within 223−848 kDa, among which the cultivars A3, A4, A5, A6, and A7 (5 out of the 8 species, 63%) exhibited smaller MW ranging within 223−343 (**Table 3**). Literature elsewhere cited that the biological activity of SP depends on their molecular size and weight. Lower molecular weight entities can be more effective in sight of immunoenhancer, as evidenced by the preparation "Biobran" manufactured by Dai-Wa Pharmaceutics (Japan) (*16*). Accordingly, one of the strategies for improving the immunobioactivity of polysaccharides is to fabricate polysaccharides having lower molecular weight analogues. Until present, there have been only few studies on the relationship between the antioxidant activity and the molecular weight (*17*).

Proximate analysis indicated that the content of carbohydrate, protein, and uronic acid in SP reached 47.8−68.3, 7.5−17.5, and 11.7−24.6%, respectively. The order (with decrease in order) was A4 (68.3%) > A2 (67.2%) > A6 (58.3%) for carbohydrate content. While for protein the order changed as A1 (17.5%) > A7 (14.0%) > A3 (12.2%); and for uronic acid, the order was A1

Table 4. Variation of Monosaccharide Composition in the Soluble Polysaccharide Fractions Obtained from Different Species of Apples[a]s

sugar (mol %)	A1	A2	A3	A4	A5	A6	A7	A8
rhamnose	6.82 ± 0.10	3.05 ± 0.01	3.23 ± 0.00	1.87 ± 0.00	3.90 ± 0.01	1.76 ± 0.00	1.18 ± 0.00	2.34 ± 0.00
fucose	13.48 ± 0.20	7.61 ± 0.10	8.08 ± 0.10	7.88 ± 0.12	6.20 ± 0.02	4.66 ± 0.00	10.35 ± 0.11	4.43 ± 0.01
ribose	5.59 ± 0.10	1.57 ± 0.00	2.43 ± 0.00	5.52 ± 0.01	2.88 ± 0.00	4.35 ± 0.00	3.87 ± 0.01	9.30 ± 0.11
arabinose	13.35 ± 0.20	25.41 ± 0.44	17.97 ± 0.40	23.85 ± 0.41	31.46 ± 0.32	20.43 ± 0.21	27.28 ± 0.44	11.75 ± 0.21
xylose	3.32 ± 0.00	2.68 ± 0.00	2.14 ± 0.00	2.89 ± 0.00	3.19 ± 0.00	1.82 ± 0.00	0.88 ± 0.00	2.18 ± 0.001
allose	19.40 ± 0.24	0.51 ± 0.00	1.15 ± 0.00	2.03 ± 0.00	0.66 ± 0.00	2.09 ± 0.00	1.29 ± 0.00	1.59 ± 0.00
talose	2.53 ± 0.00	7.57 ± 0.11	10.45 ± 0.30	17.62 ± 0.32	13.16 ± 0.24	34.36 ± 0.53	23.09 ± 0.35	45.15 ± 0.35
mannose	2.30 ± 0.00	4.22 ± 0.011	7.11 ± 0.10	3.04 ± 0.01	5.04 ± 0.01	2.36 ± 0.00	1.80 ± 0.00	2.52 ± 0.00
galactose	1.11 ± 0.00	3.59 ± 0.01	3.91 ± 0.00	2.43 ± 0.00	4.27 ± 0.01	2.26 ± 0.00	1.30 ± 0.00	1.47 ± 0.00
glucose	13.80 ± 0.21	35.18 ± 0.60	25.93 ± 0.40	20.79 ± 0.36	23.63 ± 0.42	19.71 ± 0.46	21.82 ± 0.51	9.56 ± 0.11
myo-inositol	18.30 ± 0.32	12.73 ± 0.35	17.60 ± 0.20	12.08 ± 0.27	5.61 ± 0.01	6.20 ± 0.00	7.14 ± 0.01	9.71 ± 0.12

[a] A1: *Malus domestica* "Fuji" (Japan origin). A2: *Malus domestica* "Jonagold" (Korea origin). A3: *Malus domestica* "Golden Delicious" (Japan origin). A4: *Malus domestica* "Gala" (Japan origin). A5: *Malus domestica* "Arkansas Black" (Japan origin). A6: *Malus domestica* "Fuji" (USA origin). A7: *Malus domestica* "Granny Smith" (USA origin). A8: *Malus domestica* "Red Delicious" (USA origin). Data expressed as mean ± SD from triplicate determination.

Table 5. Amino Acid Composition of the Soluble Polysaccharide Fraction Isolated from Different Species of Apples[a]

apple species	amino acid content, wt %													
	Gly	Ala	Cys	Met	Pro	Lys	Val	Leu	Ile	Phe	Tyr	His	Asp	Glu
A1	32.2 ± 3.5	7.0 ± 1.2	1.5 ± 0.3	4.3 ± 1.2	10.2 ± 2.7	0.60.73	4.7 ± 1.3	10.1 ± 1.7	3.7 ± 0.7	2.5 ± 0.5	2.6 ± 0.7	4.2 ± 1.0	1.3 ± 0.6	15.1 ± 2.7
A2	30.3 ± 4.4	5.8 ± 1.3	1.4 ± 0.2	4.5 ± 1.2	11.5 ± 2.5	0.7 ± 0.2	4.4 ± 1.8	11.3 ± 2.6	3.7 ± 0.7	2.4 ± 0.6	2.2 ± 0.4	4.1 ± 1.3	0.9 ± 0.8	16.8 ± 3.6
A3	32.1 ± 3.6	5.7 ± 12.4	1.3 ± 0.1	4.9 ± 2.3	12.2 ± 2.7	0.6 ± 0.2	4.0 ± 1.2	11.4 ± 2.7	3.7 ± 0.7	2.7 ± 0.4	2.8 ± 0.8	3.9 ± 1.2	1.3 ± 0.8	13.4 ± 3.3
A4	31.4 ± 4.3	7.0 ± 1.3	1.3 ± 0.2	3.7 ± 1.4	10.2 ± 2.3	0.5 ± 0.5	4.3 ± 2.3	10.7 ± 1.7	3.7 ± 0.7	2.8 ± 0.7	3.1 ± 0.6	4.4 ± 1.1	1.1 ± 0.6	15.8 ± 3.2.
A5	29.2 ± 2.8	6.9 ± 1.6	1.6 ± 0.3	4.8 ± 1.8	13.2 ± 2.6	0.5 ± 0.2	5.1 ± 1.9	10.6 ± 1.9	3.7 ± 0.7	1.9 ± 0.5	2.9 ± 0.9	4.1 ± 1.2	1.2 ± 0.8	14.3 ± 3.5
A6	30.6 ± 3.7	6.2 ± 1.4	1.7 ± 0.4	5.0 ± 1.8	11.2 ± 1.9	0.6 ± 0.2	5.2 ± 1.1	12.1 ± 2.7	3.7 ± 0.7	2.7 ± 0.7	2.4 ± 0.7	4.3 ± 0.8	1.0 ± 0.5	13.3 ± 2.8
A7	26.6 ± 2.7	6.7 ± 1.9	1.7 ± 0.4	4.7 ± 1.6	12.2 ± 1.8	0.7 ± 0.3	4.1 ± 1.0	13.1 ± 2.2	3.7 ± 0.7	2.2 ± 0.5	2.1 ± 0.3	4.7 ± 1.2	1.5 ± 0.6	16.0 ± 3.1
A8	30.3 ± 3.4	6.8 ± 1.7	1.6 ± 0.4	4.5 ± 1.3	9.4 ± 1.1	0.6 ± 0.4	4.8 ± 1.1	12.2 ± 2.3	3.7 ± 0.7	2.6 ± 0.6	2.2 ± 0.6	3.8 ± 1.3	1.0 ± 0.4	16.5 ± 3.0

[a] The protein contents in each individual SP sample are shown in **Table 3**.

(24.6%) > A2 (23.0%) > A4 (22.3%) (**Table 3**). As seen, apple A1 (*Malus domestica* "Fuji", Japan origin) was the most enriched in both protein and uronic acid (**Table 3**). A survey on Thai market fruits including apples performed by Chareoansiri and Kongkachuichai (*18*) indicated that the total sugar content was within 4.5−20.3 g/100 g edible portion, and the total dietary fiber content ranged from 0.6 to 11.5 g/100 g edible portion, among those apples ranked at the median (*18*), rather consistent with ours. Further analysis of SP in apples revealed the existence of 11 monosaccharides (**Table 4**). Astonishingly, data revealed the unique presence of talose, allose and fucose in the SP of apples. To our knowledge, we are the first to report this finding. In the apple SP, arabinose and glucose were the two monosaccharides mostly enriched, approaching a content over 60% (**Table 4**). The ratio of arabinose/glucose was 1.0:1.40 and 1.0:1.45 for samples A2 and A3 respectively, which approached the typical ratio of 2:3 as often defined for glucoxylans (*9*), implicating the nature of arabinoglucans (**Table 4**). Similarly, samples A4 and A5 were named glucoarabinans; sample A1, a glucoallan; sample A7, a taloarabinan; and samples A6 and A8 were designated arabinotalan (**Table 4**). More attractively, huge amount of *myo*-inositol was seen in all the apples, especially enriched in samples A1−A4 (**Table 4**). As well cited, inositol is an important signaling molecule. Phosphatidyl inositol (Ptd Ins) is phosphorylated to produce 4-phospho-phosphatidyl inositol, [Ptd Ins(4)P], and 4,5-diphospho-phosphatidyl inositol, [Ptd Ins(4,5)P_2]. By the action of phospholipase C, the latter two compounds are cleaved into 1,2-diacylglycerol and inositol (1,4,5) triphosphate, [Ins (1,4,5)P_3], which act as a crucial signal in the presence of Ca^{2+}, thus affecting cell proliferation and other cellular reactions (*19*). Recently, *myo*-inositol treatment was reported to significantly increase serum plasmalogens and decrease LDL-C, particularly in hyperlipidemic subjects with metabolic syndrome (*20*). Concomitantly, other coexisting components like uronic acid and SP may also contribute to a similar effect. As well cited, uronic acid is a potent hypolipidemic agent (*21*). The high uronic acid content in apple SP (**Table 3**) would likely elicit strong hypolipidemic bioactivity. Biochemically, the serum TG-reducing effect of diet fibers may be mediated by decreased *de novo* hepatic lipogenesis as a result of reduced activity of lipogenic enzymes involving fatty acid synthase, acetyl CoA carboxylase or ATP citrate lyase (*22*). Otherwise, the effect of diet fiber is not mediated by the inhibition of hepatic cholesterol synthesis. In addition, a potential influence of the SP on hormones regulating food intake and their implication on thermogenesis and fatty acid oxidation should not be overlooked (*23*).

In the peptidoglycans of SP, fourteen amino acids including glycine, alanine, cysteine, methionine, proline, lysine, valine, leucine, isoleucine, phenylalanine, tyrosine, histidine, aspartic and glutamic acids were present (**Table 5**), among which glycine was the most enriched, accounting for 26.6−32.2 wt %; glutamic acid was the next, ranging within 13.4−16.8 wt %. The sulfur containing amino acids, cysteine and methionine, were within 1.3−1.7 and 3.7−5.0 wt %, respectively. SP contained a relatively high level of essential amino acids like lysine, valine, leucine, isoleucine, phenylalanine (or tyrosine), histidine, and moderate proline content (9.4−13.2 wt %), underlying the high nutritious value of apples (**Table 5**).

In vivo experiment revealed that all SP samples possessed very prominent hypolipidemic bioactivity as reducing TG and TC (**Table 6**). Since the HDL-C levels were elevated (**Table 6**) and, conversely, both the LDL-C and TC levels were suppressed (**Table 6**) (*20*, *22*), the ratios LDL-C/HDL-C and TC/HDL-C were accordingly lowered (**Table 6**). Simultaneously, levels of antioxidative SOD and GPX were significantly activated (**Table 7**) ($p < 0.05$). Kinetically, in the serial attacks exerted by the *in vivo* oxidative stress, the appearance of SOD (an upstream enzyme) usually precedes catalase (a downstream enzyme), with GPX at the middle. The GPX level would be simultaneously

Table 6. Effect of Soluble Polysaccharide of Apples on Serum Lipid Parameters[a]

group	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	LDL-C/HDL-C	TC/HDL-C
C	105 ± 13 a	158 ± 25 a	123 ± 22 a	47 ± 16 a	0.38 ± 0.12 c	1.28 ± 0.15 f
A1	107 ± 14 a	165 ± 17 c	198 ± 15 g	66 ± 13 c	0.33 ± 0.08 b	0.83 ± 0.03 a
A2	114 ± 10 b	172 ± 23 e	180 ± 34 e	67 ± 26 c	0.37 ± 0.17 c	0.96 ± 0.10 c
A3	109 ± 14 a	161 ± 34 b	186 ± 22 f	54 ± 21 b	0.29 ± 0.11 a	0.87 ± 0.09 b
A4	115 ± 12 b	168 ± 21 d	178 ± 22 e	68 ± 17 c	0.38 ± 0.13 c	0.94 ± 0.12 c
A5	123 ± 17 c	167 ± 35 d	171 ± 28 d	80 ± 23 f	0.47 ± 0.15 e	0.98 ± 0.08 c
A6	137 ± 20 e	190 ± 25 h	170 ± 23 d	70 ± 18 d	0.41 ± 0.18 d	1.12 ± 0.17 e
A7	130 ± 21 d	178 ± 24 f	168 ± 28 c	70 ± 18 d	0.42 ± 0.12 d	1.06 ± 0.09 d
A8	116 ± 23 b	185 ± 27 g	172 ± 23 d	74 ± 21 e	0.43 ± 0.14 d	1.08 ± 0.11 d
H	154 ± 33 f	239 ± 44 i	160 ± 18 b	97 ± 37 g	0.60 ± 0.20 f	1.49 ± 0.15 g

[a] Data are expressed as mean ± SD from triplet experiments. ($n = 8$). The serum of each animal analyzed separately in triplicate for a total of 24 measurements per group. Data in the same column with different superscripts are significantly different from each other ($p < 0.05$). TG: serum triglyceride. TC: serum total cholesterol. HDL: high density lipoprotein. LDL: low density lipoprotein. Groups C and H: Group C fed with normal diet, group H fed with high fat diet; both groups were without administration of any of the test samples.

Table 7. Effect of the Soluble Polysaccharides of Apples on Serum Antioxidative Enzyme Activity in Hamsters[a]

group	SOD (U/L)[b]	GPX (U/L)[c]
C[d]	660 ± 11 a	92 ± 4 d
A1	789 ± 25 c	86 ± 4 b
A2	880 ± 23 d	96 ± 3 f
A3	790 ± 27 c	99 ± 6 f
A4	988 ± 20 f	87 ± 6 b
A5	1335 ± 41 g	95 ± 5 e
A6	1318 ± 49 h	91 ± 7 c
A7	898 ± 40 d	87 ± 6 b
A8	979 ± 27 e	88 ± 6 b
H[d]	732 ± 33 b	82 ± 3 a

[a] Data are expressed as mean ± SD from triplet experiments. ($n = 8$). Data in the same column with different superscripts are significantly different from each other ($p < 0.05$). The serum of each animal analyzed separately in triplicate for a total of 24 measurements per group. [b] SOD: superoxide dismutase: One unit of SOD is defined as the capacity of 50% inhibition on the auto-oxidation rate of pyrogallol. [c] GPX: glutathione peroxidase: 1 unit of GPX will cause the formation of 1 μmol of $NADP^+$ from NADPH per min at pH 8.0 at 25°C . Extinction coefficient for NADPH is 0.00622 μM^{-1} cm^{-1} at 340 nm. [d] Groups C and H: Group C fed with normal diet and group H fed with high fat diet to serve as the normal and high fat control, respectively.

affected by these two antioxidative enzymes, hence the GPX level was damped as a consequence of balance between these two parameters.

Literature indicated that high levels of soluble dietary fiber improved mucosal integrity and functionality. Soluble rye fiber slowed the velocity of carbohydrate absorption due to the different consistency of the dietary fibers utilized. Intake of arabinoxylan or pectin retained carbohydrate oxidation at higher efficiency during the consumption period (*24*). In contrast, a low viscosity soluble fiber fruit juice supplement failed to lower cholesterol in hypercholesteremic subjects (*25*). The tamarind xyloglucans had been shown to block UV-activated phosphorylation of SAPK/JNK protein but have no effect on p38 phosphorylation. These results indicated that animals, like plants, are able to take advantage of the carbohydrate utilization rate to regulate responses to environmental stimuli (*7*).

Literature elsewhere also pointed out that certain beneficial effects of dietary fibers are mediated by short-chain fatty acids that are produced during anaerobic fermentation in the colon. Butyrate was suggested to act as a chemopreventive metabolite which prevented the occurrence of colorectal cancer, one of the abundant cancers in the highly developed Western countries (*4*). Recently, apple pectin and apple juice extracts were shown to inhibit histone deacetylase (HDAC) (*26*). Butyrate is considered to be an important nutrient of colon mucosa, has been shown to trigger differentiation and apoptosis of colon-derived cells in culture, and is the most relevant HDAC inhibitor (*26*). Previously, Barth et al. demonstrated cloudy apple juice (CAJ) to be more effective than apple polyphenols (AP) and apple juice derived cloud fraction (CF) in a rat model of colon carcinogenesis (*27*). The CAJ contained a total AP and a CF consisting of proteins, fatty acids, polyphenols, and cell wall polysaccharides. CF was thus identified to be more bioactive (*28*).

In reality, the polyphenolics may contribute to the biochemical effects mentioned in the above (*29*). Tsao's study demonstrated five major polyphenolic groups comprising a total of 16 identified individual compounds, among which the dihydroxycinnamic acid esters, phloretin glycosides and flavan-3-ols were present in both flesh and peel, whereas quercetin glycosides were exclusively found in the peel (*30*). The cyanidin 3-galactoside was uniquely found in red apple peels only (*29*). On average, 46% of the polyphenolics in whole apples were present in the peels, i.e. essentially all of the flavonols (quercetin derivatives) were present in the peels (*31*), consistent with Tsao's finding (*30*). It implicated that 50% of polyphenolics were still contained in the flesh. Odbayar et al. demonstrated phenolic compounds possessing potent suppressive effect on lipogenesis (*32*).

Conclusively, the yield of SP of apples ranges within 0.43−0.88%, having a mean molecular weight 223 to 848 kDa. Referring to the monosaccharide composition, 50% of SP is arabinoglucan (or glucoarabinan); 37.5% is arabinotalan (or taloarabinan), and the remaining 12.5% belongs to alloglucan. The hypolipidemic effect of peeled apples is most likely attributable to its high content of SP, *myo*-inositol and uronic acid. However, in dealing with the nutritive value of whole apples, the potential contribution of the polyphenolic compounds in SP still cannot be ruled out. A number of studies suggested that there is a "synergism" between dietary fiber and phenolics in controlling the risk of cardiovascular disease. In fact, it was found that apple pectin and the polyphenolic fraction lowered plasma cholesterol and triglycerides and were more effective together than either apple pectin alone or apple phenolics alone (*33*).

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