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Physicochemical Properties of Water-soluble Polysaccharide Enriched Fractions of Adlay and Their Hypolipidemic Effect in Hamsters

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ABSTRACT

To investigate the physicochemical propertites of the water-soluble polysaccharide enriched fractions of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) and their hypolipidemic effects, we fed the enriched fraction adlay water-soluble dietary polysaccharide (AWSP) to hamsters to evaluate the effects of AWSP on serum and hepatic cholesterol and triglyceride levels. The AWSP used was extracted from dehulled adlay and its high- and low-molecular weight fractions (AWSPH and AWSPL, respectively) and prepared by 40% ethanol concentration fractionation. Hamsters fed with AWSP showed serum / hepatic total cholesterol, triglyceride and serum LDL cholesterol levels significantly lower than those in the cellulose control group. We found higher serum hypolipidemic activities associated with AWSPH than with AWSPL, which were correlated with AWSPH's higher molecular weight and water-holding capacity. Fecal lipids data supported our theory that the hypolipidemic activities of AWSPH decrease cholesterol absorption in the intestines. We found ASDFL to be relatively more fermentable in cecum and to have higher hepatic hypolipidemic activities than AWSPH, which correlated with the amount of short-chain fatty acid in cecum. The apparent molecular-weights of AWSPH and AWSPL were 14.5 and 8.7×10^4 Daltons, respectively.

Key words: adlay, water-soluble polysaccharide, physicochemical property, hypolipidemic effect, hamster

INTRODUCTION

Adlay (Job's Tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a cultivated grain crop that has long been consumed in ancient China for its nutrition and used as a herbal medicine. The ancient Chinese medicinal book *Pen-Tsao-Kang-Mu* describes its pharmacological properties in the treatment of numerous maladies including warts, chapped skin, and rheumatism[1], whereas our laboratory has previously reported on Adlay's various physiological effects, such as desmutagenic[2], anti-tumor[3,4], anti-allergic[5,6] and hypoglycemic[7]. Adlay is cultivated widely in Taiwan, China and Japan, where it is widely appreciated as a healthy food supplement.

Clinical and animal studies have shown that dehulled adlay, polished adlay, defatted adlay, adlay bran, adlay lipids and water extract of adlay help improve lipid metabolism[8-11]. Adlay consumption effectively decreases serum cholesterol, triglyceride, and low density lipoprotein (LDL) cholesterol, increases high density lipoprotein (HDL) cholesterol, lowers liver lipids, prevents the causes fatty liver[12,13], and increases excretion of lipids[14]. Most studies hypothesize that the effective hypolipidemic components of adlay are dietary fiber, oil and protein[10-14]. Many cereal grains, like oats, barley, and rice, also have hypolipidemic effects, with dietary fiber being attributed as their main effective component[15-17]. The physicochemical properties of dietary fiber, e.g., molecular weight, fermentability, water-holding capacity, bile acid-binding ability and fecal-bulking ability, dictate the hypolipidemic effects which may govern ingestion of a particular fiber type[18].

There remains little information on the above physicochemical properties of AWSP, the water-soluble polysaccharide of adlay, even without its physiological effects on lipid metabolism. Therefore, this study was developed to investigate the physicochemical properties of AWSP and its fractions, AWSPH (40% enthanol–insoluble) and AWSPL (40% enthanol–soluble). Samples were prepared by 40% ethanol fractionation and used to evalute the hypolipidemic effects in hamsters fed diets supplemented with cholesterol.

MATERIALS AND METHODS

I. *Preparation of Water-soluble Polysaccharide Enriched Fractions of Adlay*

Dehulled adlay of the variety Taichung Shuenyu No. 4 (TCS4) (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) was purchased in Taichung, Taiwan for this study. The water-soluble polysaccharide enriched fractions were prepared of adlay as follows: Dehulled adlay was soaked in cold water (adlay/water = 1/3, w/v) at 15°C for 12 hr, after which it was wet milled, filtrated and settled to provide both insoluble residue and supernatant fractions. The

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supernatant fraction was then concentrated with a vacuum evaporator and adjusted to an 80% ethanol concentration to allow the water-soluble polysaccharide fraction (AWSP) to precipitate. After settling in a refrigerator (6°C) overnight, we collected the precipitate using a centrifuge (1500 ×g for 15 min). Furthermore, by using fractional precipitation with 40% ethanol, AWSP was redissolved in distilled water to get 2 separated fractions: AWSPH and AWSPL. After settling overnight, the precipitate was collected using a centrifuge (1500 ×g for 15 min) and called adlay water-soluble polysaccharide fraction 1 (AWSPH) (40% enthanol–insoluble). The supernatant was then concentrated with a vaccum evaporator to prepare adlay water-soluble polysaccharide fraction 2 (AWSPL) (40% enthanol–soluble).

The compositions of AWSP, AWSPH and AWSPL are summarized in Table 1. Moisture, crude fat, crude protein and ash were measured by the AOAC method[19]. Soluble, insoluble and total dietary fiber contents were analyzed using the method used by Prosky *et al.*[20].

II. *Analysis of the Physicochemical Properties of Water-soluble Polysaccharide Fractions of Adlay*

(I) *Determination of Apparent Molecular Weight*[21]

The AWSP, AWSPH and AWSPL samples were assessed using high performance size-exclusion chromatography (HPSEC). The HPSEC system included an SSI single pump (Scientific Systems, Inc., State College, PA, USA), a column oven (Super Co-150, Enshine, Tainan, Taiwan) equipped with a Rheodyne injector (Cotati, PA, USA), a 100-µL sample loop, and an OPTILAB DSP interferometric refractometer (P10 cell, 690 nm, Wyatt) with temperature controlled to 35°C. We performed HPSEC using 3 columns (7.8 × 300 mm) (PW_{XL} 4000, PW_{XL} 3000, PN_X 2500, Tosho Tokyo, Japan) in series running at 60°C. The mobile phase was 0.3 N $NaNO_3$ with 200 mg/L NaN_3 at a rate of 0.5 mL/min. Samples were prepared at 0.1% (w/v) concentration and filtered (0.45 µm) before being injected (100 µL). The pullunans (P-5, P-10, P-20, P-50, P100, P200, P400, P800, Shodex®) were used as calibration standards.

(II) *Determination of Water-holding Capacity*[22]

The water-holding capacity of AWSP, AWSPH and AWSPL were measured by soaking samples (1 g) in distilled water (20°C) for 24 hr and then centrifuging them at 3000 × g for 15 min. The water-holding capacity was expressed as grams of water held by one kilogram of dry sample.

III. *Animal Experiment*

Thirty-two male Golden Syrian hamsters (6 weeks old) were purchased from the Animal Breeding and Research Center of the National Science Council of the Republic of China (Taipei, Taiwan) and were fed a commercial rodent diet (Hope Farms, Woerden, The Netherlands) for 2 weeks. The hamsters were subsequently divided on the basis of equivalent body weight into 4 groups of 8. The 4 groups were then begun on test diets. The control diet (C) was based on the AIN-76 diet, with the following composition (g/100 g diet): corn starch, 59.25; casein, 20; corn oil, 10; cellulose, 5; AIN-76 mineral mix, 4; AIN-76 vitamin mix, 1; DL-methionine, 0.3; choline choloride, 0.2; cholesterol, 0.25. For the other 3 groups fed on an adlay water-soluble polysaccharide diet (AP, APH and APL), cellulose (5 g/100g diet) was substituted with an equivalent amount of the total dietary fiber of 6.68 g AWSP (AP); 6.17 g AWSPH (APH); and 8.04 g AWSPL (APL), respectively (Table 2). The hamsters had free access to food and tap water and were fed on these diets for 28 days. Actual feed consumption was recorded daily and body weights were monitored on a weekly basis. The animal room was temperature-controlled (22 ± 2°C) and had a 12-hr light-dark cycle. At the end of the experiment, the hamsters were killed after fasting for 12 hr. Blood samples were collected and centrifuged at 3000 ×g for 30 min to obtain the serum for serum lipids

Table 1. Composition of water-soluble dietary fiber-rich fractions of adlay*

Component (g/100 g, dry weight)	AWSP	AWSPH	AWSPL
Moisture	4.4 ± 0.2	2.3 ± 0.3	3.7 ± 0.2
Crude fat	1.8 ± 0.2	1.9 ± 0.2	0.2 ± 0.1
Crude protein	6.5 ± 0.3	7.4 ± 0.4	2.9 ± 0.3
Ash	3.6 ± 0.1	2.8 ± 0.2	4.5 ± 0.4
Total dietary fiber	74.8 ± 2.1	81.5 ± 6.1	62.2 ± 3.7
Soluble dietary fiber	71.9 ± 4.0	78.8 ± 2.2	61.8 ± 2.9
Insoluble dietary fiber	2.9 ± 1.8	2.3 ± 3.4	0.4 ± 0.1
Nitrogen-free extract**	8.9 ± 1.1	4.1 ± 1.5	26.5 ± 3.0

*Data are expressed as mean ± S.D. (n = 3).

**Nitrogen-free extract = 100–(moisture + crude fat + crude protein + ash + total dietary fiber).

Table 2. Composition of the various experimental diets

Ingredient	Feed composition* (g/100 g, dry weight)			
	C	AP	APH	APL
Fiber				
Cellulose	5			
AWSP		6.68		
AWSPH			6.17	
AWSPL				8.04
Casein	20	19.57	19.54	19.77
Corn starch	59.25	58.65	59.0	57.12
Corn oil	10	9.88	9.88	9.98
AIN-76 mineral mix	4	4	4	4
AIN-76 vitamin mix	1	1	1	1
DL-Methionine	0.3	0.3	0.3	0.3
Choline choloride	0.2	0.2	0.2	0.2
Cholesterol	0.25	0.25	0.25	0.25

*The C dietary group represents the control diet, which contained cellulose as the fiber source. The AP diet contained AWSP instead of cellulose as the fiber source. The APH diet contained AWSPH instead of cellulose. The APL diet contained AWSPL instead of cellulose.

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analysis. The liver was removed, washed with saline, blotted to remove excess solution and blood, weighed and stored at -70°C for liver lipids analysis. Cecal samples were collected after cecums were excised and the contents were extruded, weighed and stored at -70°C for short chain fatty acid determination. Fresh fecal samples were collected three days prior to sacrifice, and then freeze-dried and stored at -70°C for fecal total lipid and steroid measurements.

IV. *Lipids Analysis*

(I) *Determination of Serum Lipid Levels*

Using commercially available kits, levels of serum total (Catalog No. 402, Sigma Chemical Co., St. Louis, MO, USA), HDL (Catalog No. 352-4), LDL cholesterol (Catalog No. 352), and triglyceride (Merckotest 14354, Merck, Germany) in the serum samples collected were measured enzymatically.

(II) *Determination of Hepatic Cholesterol and Triglyceride Levels*

Using the method of Folch *et al.*[23], we extracted hepatic cholesterol and triglyceride from 1~2 g of liver with a chloroform/methanol mixture (2/1, v/v). The concentration of hepatic total cholesterol and triglyceride in the lipids extracted were determined by the method of Carr *et al.*[24].

(III) *Determination of Cecal Short-chain Fatty Acid (SCFA) Levels*[25]

Five quantities (v/v) of physiological saline solution (0.9% NaCl + 0.02% NaN_3) were added to the weighed samples, and the samples were homogenized with a Waring blender for 3 min. The mixtures were then centrifuged at 11,000 ×g (4°C) for 15 min. One milliliter of the supernatant was mixed with 10 mL of H_2SO_4 (50%, v/v) and extracted with 1 mL of diether ether. After thorough shaking, the samples were centrifuged at 11000 ×g (4°C) for 5 min. The ether layer was pipetted out, and a small amount of anhydrous magnesium sulfate was added to remove water. The ether samples were analyzed with gas chromatography (GC) (Hewlett-Packard 5890 GC with flame ionation detection and a Stabilwax-DX column (Restek, No. 10823, length 30 m, I.D. 0.25 mm) for short-chain fatty acids. The column temperature was kept at 145°C. The injector and detector were maintained at 250°C. Helium was used as the carrier gas. Pure acetic acid, propionic acid and *n*-butyric acid (Sigma) were co-injected as standards. The method used to calculate SCFA content followed previously published practice.

(IV) *Determination of Fecal Lipid Levels*

Feces were ground into fine power and extracted with a chloroform/methanol (2/1, v/v) mixture. Fecal total lipids were determined gravimetrically by evaporating off the organic solvent in the fecal lipid extract. Fecal steroids were measured using a method described by Chezen and Story[26]. Briefly, sterols were extracted from a 0.5 g fecal sample with 5 ß-cholanic acid and 5 ß-cholestane as internal standards for acidic steroids and neutral steroids, respectively. After deconjugating bile acids with cholyglycine hydrolase, neutral steroids were extracted with petroleum ether and bile acids with diethyl ether and ethyl acetate, respectively. Quantification of trimethylsylyl ethers of neutral steroids involved gas liquid chromatography (Hewlett Packard, Cincinnati, OH) with a 30-m DB1701 capillary column (J&W Scientific, Folsom, CA, USA). Bile acids were further purified using octadecylsilane-bonded silica cartridges (Millipore, Milford, MA, USA) and quantified as trimethylsylyl ethers with a 30-m DB5 capillary column (J&W Scientific).

V. *Statistical Analysis*

Data represent the mean ± S.D. and differences from the treatment group mean were determined by one-way ANOVA followed by Duncan's multiple range test using a Statistical Analysis System (SAS Instute, Cary, NC)[27]. A value of $p < 0.05$ was considered statistically signficant.

RESULTS AND DISCUSSION

I. *The Physicochemical Properties of Water-soluble Polysaccharide Enriched Fractions of Adlay*

Table 1 shows that adlay water-soluble polysaccharide (AWSP) and its fractions (AWSPH and AWSPL) are all rich in water-soluble dietary fiber. Total dietary fiber, soluble dietary fiber and insoluble dietary fiber in each is 74.8 g, 71.9 g, and 2.9 g for AWSP; 81.5 g, 78.8 g and 2.3 g for AWSPH; and 62.2 g, 61.8 g and 0.4 g for AWSPL (g/100 g, dry weight). The AWSPL fraction also contains high nitrogen-free extract (26.5%). While we believe this extract may be the result of a short-chain polysaccharide, further investigation is advised.

The physicochemical properties of water-soluble polysaccharide enriched fractions of adlay are shown

Table 3. The apparent molecular weight and water-holding capacity of adlay water-soluble polysaccharise enriched fractions*

Physicochemical property	Cellulose	AWSP	AWSPH	AWSPL
Apparent molecular weight (× 10^4 Daltons)	ND**	12.4	14.5	8.7
Water-holding capacity (g/g)	2.2 ± 0.1^a	3.5 ± 0.1^c	4.1 ± 0.2^d	3.2 ± 0.1^b

*Data are expressed as mean ± S.D. ($n = 3$). Values in the same row with different superscript letters are considered significantly different ($p < 0.05$) .

**Not determined.

in Table 3. The apparent molecular weight of AWSP, AWSPH and AWSPL are estimated at 12.4, 14.5 and 8.7 $\times 10^4$ Daltons, respectively. We believed these 3 fractions might be amylopectin-like polysaccharides. Yamada *et al.*[28] reported that anti-complementary and anti-tumor polysaccharides extracted from coix seeds were characterized to be amylopectin-like glucan mixtures. These glucans contained (1→4) linked α-D-glucans to which glucosyl side chains were attached at O-6 of the main chain. Apparent molecular weights were 7.0 $\times 10^4$ and 16 $\times 10^4$ Daltons.

The molecular weight of ß-glucan is thought to play an important role in the cholesterol-lowering activity of barley grain and ß-glucan concentrate prepared from oats. Wilson *et al.*[29] demonstrated a similar cholesterol-lowering effect in hamsters of oat ß-glucan with molecular weights from 13.6 to 165 $\times 10^4$ Daltons. We found both that the water-holding capacity of each of the 3 water-soluble polysaccharide enriched fractions of adlay was significantly higher than that of cellulose (Table 3) and that there exists significant differences between the water-holding capacities of the 3 fractions. AWSPH showed the highest water-holding capacity (4.1 g/g), followed by AWSP (3.5 g/g) and AWSPL (3.2 g/g).

II. *Effect of Adlay Water-soluble Polysaccharide Enriched Fractions on Hamster Serum Lipids*

The 4 diets (described in Table 2) were fed to the hamsters as shown in Table 4. Food intake did not differ significantly between the 4 groups over the course of the 4-week study (6.1, 6.0, 6.1 and 6.2 g/day, respectively, for groups C, AP, APH, and APL). Body weight gains within the 4 groups were also similar (0.71, 0.75, 0.72 and 0.80 g/day, respectively).

Serum triglyceride and total cholesterol levels for hamsters fed different diets are shown in Table 4. The serum triglyceride, total cholesterol and LDL-cholesterol levels in hamsters fed the AP (317, 228, 41 mg/dL) diet were significantly lower than in those on the C (431, 254, 64 mg/dL) diet. Water-soluble fiber sources, such as ß-glucan and pectin, appeared to have plasma cholesterol lowering effect. However, water-insoluble fiber sources, such as cellulose and lignin, did not appear to have a hypocholesterolemic effect[30,31]. Chung *et al.*[11] demonstrated previously that plasma cholesterol levels fell significantly in rats fed a coix lipid-free residue diet, compared to those fed a cellulose diet. Their findings suggested that the hypocholesterolemic effect of the coix might result from the presence of coix dietary fiber. Our results demonstrated adlay water-soluble dietary fiber to be a hypolipidemic component.

Park *et al.*[14] have suggested several possible mechanisms by which coix triggers lower plasma lipid levels in rats fed high fat diets. These include decreased cholesterol absorption activity, enhanced fecal lipid excretion, and suppression of cholesterol synthesis in the liver. As shown in Table 5, wet fecal weight, fecal total lipids, neutral cholesterol and bile acid excretion in hamsters fed the 3 water-soluble polysaccharide enriched fractions (AWSP, AWSPH and AWSPL) of adlay diets (AP, APH and APL) were all significantly higher than in hamsters fed the cellulose (C) diet. Gallaher *et al.*[32] previously remarked that water-soluble fibers are always more viscous than water-insoluble fibers and that the higher the apparent molecular weight of a water-soluble fiber the greater its viscosity. A high viscosity level associated with soluble fiber has also been postulated to impair micelle formation, which further reduces fatty acid and cholesterol absorption[33,34]. Therefore, we postulate that the mechanism that results in a lowering of serum lipid levels in hamsters (Table 3) is related to the water-soluble polysaccharide enriched fractions of adlay which decrease cholesterol absorption in

Table 4. Food intake, body weight gain, serum triglyceride and cholesterol levels for hamsters fed different diets*

Item	C	AP	APH	APL
Food intake (g/day)	6.1 ± 0.8	6.0 ± 0.5	6.1 ± 0.5	6.2 ± 0.7
Body weight gain (g/day)	0.71 ± 0.18	0.75 ± 0.23	0.72 ± 0.14	0.80 ± 0.21
Triglyceride (mg/dL)	431 ± 36^{c}	317 ± 46^{ab}	284 ± 42^{a}	330 ± 32^{b}
Total cholesterol (mg/dL)	254 ± 11^{c}	228 ± 7^{ab}	204 ± 10^{a}	231 ± 9^{b}
HDL cholesterol (mg/dL)	142 ± 6	147 ± 10	139 ± 9	143 ± 7
LDL cholesterol (mg/dL)	64 ± 5^{c}	41 ± 7^{ab}	38 ± 5^{a}	45 ± 7^{b}

*Data are expressed as mean ± S.D. for 8 hamsters per dietary group. Values in the same row with different superscript letters are considered significantly different ($p < 0.05$).

Table 5. Fecal total lipids, neutral cholesterol and bile acid excretion for hamsters fed different diets*

Item	C	AP	APH	APL
Food intake (g/day)	6.1 ± 0.8	6.0 ± 0.5	6.1 ± 0.5	6.2 ± 0.7
Fecal weight (g wet feces /day)	1.62 ± 0.14^{a}	1.88 ± 0.10^{bc}	1.95 ± 0.11^{c}	1.81 ± 0.10^{b}
Total lipids (mg/g dry feces)	30.3 ± 5.1^{a}	51.5 ± 3.4^{c}	54.0 ± 2.4^{c}	44.5 ± 3.5^{b}
Neutral cholesterol (mg/g dry feces)	5.8 ± 1.9^{a}	18.0 ± 2.4^{c}	20.4 ± 4.0^{c}	12.5 ± 2.1^{b}
Bile acid (mg/g dry feces)	2.0 ± 0.7^{a}	8.2 ± 1.1^{c}	9.3 ± 0.6^{c}	5.9 ± 1.0^{b}

*Data are expressed as mean ± S.D. for 8 hamsters per dietary group. Values in the same row with different superscript letters are considered significantly different ($p < 0.05$).

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the intestine and enhance the excretion of fecal lipids.

Moreover, variations in the serum hypolipidemic effect amongst different water-soluble polysaccharide enriched fractions of adlay (AWSP, AWSPH and AWSPL) may be related to their different physicochemical properties. The fraction showing the highest molecular weight and water-holding capacity (Table 3) also had the highest fecal weight and fecal lipids excretion values (Table 5) and lowest serum triglyceride, total cholesterol and LDL-cholesterol levels (Table 4). Yang and Tsai[13] showed that, while levels of serum total lipids, triglyceride, cholesterol and LDL cholesterol were lower in hamsters fed dehulled adlay than in hamsters fed oats (although oats had a higher content of soluble dietary fiber than dehulled adlay), there was no significant difference in the degrees by which serum lipids were lowered. They suggested that this may be related to the unique physicochemical properties of the 2 grains. AWSPH showed higher serum hypolipidemic activity than AWSPL. One possibility is that higher serum hypolipidemic activity correlates to higher molecular weight and water-holding capacity (Tables 3, 4 and 7). Therefore, our results demonstrated that the physicochemical properties of adlay water-soluble polysaccharides indeed influenced the hypolipidemic effects in hamster serum lipids.

Serum HDL cholesterol levels did not differ significantly in hamsters fed the 4 diets. This finding is similar to several studies that reported that HDL cholesterol levels remained stable in rats fed water-soluble fiber from ß-glucan and pysillum as well as water-insoluble fiber from cellulose, oats and barely[35,36]. Yang and Tsai[13] demonstrated that HDL cholesterol levels increased in hamsters fed diets containing dehulled or polished adlay. Aoki and Tuzihara revealed that concentrations of HDL cholesterol rose in 3 male rat strains (Sprague-Dawley, SHR, and Wister Kyoto) fed semipolished Hatomugi and polished rice plus 3.9% Hatomugi dietary fiber[9]. We attributed the higher HDL cholesterol level in hamsters fed adlay to dietary factors other than the adlay water-soluble polysaccharide.

III. *Effect of Adlay Water-soluble Polysaccharide Enriched Fractions on the Hepatic Lipids in Hamsters*

Our results (Table 6) revealed no significant difference in liver weight among the 4 groups of hamsters fed different diets. Previous studies on various animals indicated that weight and appearance variations in the liver of animals fed different diets can be attributed to differing amounts of cholesterol and oil in the diets[37,38]. As the fiber source was the only ingredient variable in our test diets (Table 2), we would, therefore, expect little difference in the liver weights.

As compared to the control diet (C), the AP, APH and APL diets containing adlay water-soluble polysaccharide enriched fractions significantly reduced hepatic total cholesterol and triglyceride levels (Table 6). Hamsters fed dehulled adlay or polished adlay showed decreased levels of total liver lipids, triglyceride and cholesterol, which may prevent the causes of fatty liver. This suggests that the above cause of lowered hepatic lipids levels was related to the water-soluble dietary fiber of adlay. Masur *et al.*[39] and Lo *et al.*[40] previously reported that water-soluble fractions in soybean fiber appear to play a positive role in the lowering of liver cholesterol levels in rats and rabbits. Other previous findings[41] indicated that soluble fibers from oats, wheat and barley may increase digesta viscosity and the thickness of the unstirred layer in the small intestine. These soluble fibers show that gelling and thickening properties can decrease cholesterol and triglyceride absorption, reabsorb bile acids, increase fecal bile acid excretion, and accelerate the synthesizing of hepatic cholesterol into bile salts. Therefore, these fractions may lower serum and hepatic lipid levels because the reduction in hepatic cholesterol levels (Table 6) paralleled that of serum cholesterol (Table 4) and also showed increased fecal lipid excretion (Table 5). We consider that the reduction of hepatic cholesterol in hamsters was related to adlay water-soluble polysaccharides due to

Table 6. Hepatic cholesterol and triglyceride levels for hamsters fed different diets*

Item	C	AP	APH	APL
Liver weight/body weight (g/100g)	4.4 ± 0.3	4.3 ± 0.4	4.5 ± 0.3	4.2 ± 0.3
Total cholesterol (mg/g liver)	22.3 ± 2.1^{c}	14.7 ± 1.6^{ab}	15.1 ± 2.3^{b}	12.0 ± 1.8^{a}
Triglyceride (mg/g liver)	16.5 ± 1.8^{b}	11.6 ± 1.5^{a}	12.9 ± 2.2^{a}	10.5 ± 2.0^{a}

*Data are expressed as mean ± S.D. for 8 hamsters per dietary group. Values in the same row with different superscript letters are considered significantly different ($p < 0.05$).

Table 7. Types and Quantities of short-chain fatty acids (SCFA) in the cecum of hamsters fed different diets*

SCFA (μmol/g wet cecal content)	C	AP	APH	APL
Acetic acid	54.5 ± 4.0^{a}	84.8 ± 4.5^{b}	88.1 ± 2.7^{b}	98.4 ± 4.5^{c}
Propionic acid	14.3 ± 2.7^{a}	29.4 ± 3.1^{b}	30.5 ± 2.3^{b}	38.9 ± 5.0^{c}
Butyric acid	10.4 ± 1.5^{a}	16.3 ± 1.9^{bc}	18.2 ± 1.4^{c}	13.4 ± 2.0^{b}
Total SCFA**	79.2 ± 4.2^{a}	130.4 ± 3.9^{b}	136.8 ± 3.5^{b}	150.7 ± 4.7^{c}

*Data are expressed as mean ± S.D. for 8 hamsters per dietary group. Values in the same row with different superscript letters are considered significantly different ($p < 0.05$).

**Total SCFA = acetic acid + propionic acid + butyric acid.

reduced exogenous cholesterol absorption and increased bile acid excretion.

Moreover, levels of acetic acid, propionic acid, butyric acid and total SCFA in hamsters fed diets (AP, APH and APL) containing adlay water-soluble polysaccharide enriched fractions (AWSP, AWSPH and AWSPL) were significantly higher than in hamsters fed on the cellulose (C) diet (Table 7). These results are consistent with the findings of Chiang *et al.*[25]. A previous study showed that total cecal SCFA levels were significantly higher in animals consuming diets that contained 20% and 40% dehulled adlay compared with those fed a control cellulose diet. Fermentability is another property typically associated with soluble fiber. Soluble fibers vary widely in their fermentability. Some types, such as psyllium or xanthum gum, are as poorly fermentable as insoluble fibers (e.g. cellulose). Some types, such as pectin or β-glucan, are fermentable and produce high levels of SCFA in the intestines. Uptake of these fatty acids by the coloncytes has been associated with a number of effects including increased cell proliferation rates, enhanced sodium and fluid uptake, and inhibition of hepatic cholesterol synthesis. It has been proposed that propionic acid may regulate plasma cholesterol through the inhibition of hepatic cholesterol[42,43]. Evaluation of data obtained in the present experiment revealed a strong negative correlation between hepatic cholesterol and cecal propionate (Tables 6 and 7). Compared with AWSPH, AWSPL was found to be significantly more fermentable in the cecum and showed higher hepatic hypolipidemic activity (Table 6). It is conceivable that these activities were more related to the relative amounts of short-chain fatty acids, especially higher propionic acid, in the cecum (Table 7) than to the amount of fecal lipids excreted (Table 5).

CONCLUSIONS

We found that water-soluble polysaccharide enriched fractions of adlay have a hypolipidemic effect on lipid metabolism in hamsters. The water-soluble polysaccharide of adlay is associated with lower serum triglyceride, total cholesterol and LDL cholesterol concentrations, lower hepatic triglyceride and total cholesterol concentration, as well as higher cecal SCFA concentration, fecal lipid, neutral cholesterol and bile acid excretions. These effects may result from changes in cholesterol absorption and /or synthesis due to variations in cecal fermentation and increases in lipid, neutral cholesterol and bile acid levels in fecal excretions. On the other hand, hypolipidemic effects were also influenced by physicochemical property variations. AWSPH showed a higher serum hypolipidemic activity than AWSPL, which correlated with AWSPH' s higher molecular weight and water-holding capacity. AWSPL showed higher hepatic hypolipidemic activities than AWSPH, which correlated with AWSPL's higher fermentability.

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