DOI 10.1002/mnfr.200500060 Mol. Nutr. Food Res. 2005, *49*, 786–790

Effects of the insoluble fiber derived from *Passiflora edulis* seed on plasma and hepatic lipids and fecal output

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The influence of the insoluble fiber-rich fraction (FRF) prepared from defatted *Passiflora edulis* seed, a potential fiber source, on plasma and hepatic lipids and fecal output were investigated in hamsters fed a hypercholesterolemic diet containing 5% insoluble FRF. The results showed that the consumption of insoluble FRF diet relative to cellulose diet could effectively ($P < 0.05$) decrease the levels of serum triglyceride, serum total cholesterol, and liver cholesterol, and increase ($P < 0.05$) the levels of total lipids, cholesterol, and bile acids in feces. The consumption of insoluble FRF also increased ($P < 0.05$) the fecal bulk and moisture. The marked cholesterol- and lipid-lowering effects of insoluble FRF might be partly attributed to its ability to enhance the excretion of lipids and bile acids *via* feces. Our results suggested that insoluble FRF could be a potential hypocholesterolemic ingredient for fiber-rich functional foods, but some further researches in humans may be needed to confirm its benefits.

Keywords: Bile acids / Cholesterol / Fiber-rich fraction / *Passiflora edulis* / Passion fruit seed

Received: April 29, 2005; revised: May 19, 2005; accepted: May 25, 2005

1 Introduction

Many recent findings have demonstrated an important role of insoluble dietary fiber in lowering the risk of cardiovascular disease, gastrointestinal disease, colon cancer, and obesity [1–5]. Insoluble fibers of some fruits, vegetables, and pomace might promote a significant decrease in blood cholesterol concentration [6–8]. The mechanisms whereby dietary fibers lower the concentrations of serum lipids might include alterations in dietary intake, conversion of bile acids, reduced cholesterol biosynthesis, and reduced absorption of lipid, cholesterol, and bile acid [7, 9–11]. The importance of dietary fibers to health has therefore encouraged food scientists to continue searching for new fiber sources as functional ingredients for food applications [12].

Passion fruit (*Passiflora edulis*) is a popular tropical fruit throughout the world, and is usually used for juice production in Asia. After juice extraction, many thousand tons of edible albuminous seeds as agricultural byproducts are produced. The raw seed is rich in aromatic crude oil (24.5 g/100 g of raw seed) which has high level of linoleic acid (∼65%) [8, 13]. Our previous study [13] revealed that the insoluble fiber-rich fraction (FRF) derived from the defatted passion fruit seed is the predominant component in the defatted seed (up to 93.3 g/100 g), and has desirable characteristics and physicochemical properties to be a potential fiber source. Since the fiber-rich passion fruit seed is available in large quantity as a byproduct in juice production, the insoluble FRF could be exploited as a good source of low-calorie functional ingredients. In addition to the physicochemical properties, the study of the physiological effects of dietary fibers is also important for the understanding of their potential functions in the human body as well as food applications.

The present study was to investigate the influence of insoluble FRF derived from defatted passion fruit seed on the plasma and hepatic lipids and fecal output in hamsters fed hypercholesterolemic diets. Furthermore, the relationship between the physiological functions and physicochemical properties of the insoluble FRF was also interpreted.

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Abbreviations: FRF, fiber-rich fraction; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein

2 Materials and methods

2.1 Separation of insoluble FRF

Seed samples of passion fruit (hybrid, Tai-Nong-1) collected from the CHIA-MEEI (Taiwan) Food Industrial Corp. were finely ground to 0.5 mm in size. After defatting with petroleum ether (1 : 4 w/v) for four times, the residual lipid content in the defatted sample was below 0.01 g/100 g. According to the method of Chau and Huang [13], insoluble FRF was prepared by homogenizing the defatted sample in cold distilled water (seed : water 1 : 10 w/v) using the Osterizer (Sunbeam-Oster, Niles IL, USA) at the "Hi" speed for 1 min. After filtration, the insoluble FRF collected was washed with cold distilled water, and dried by solvent exchange and air at 30°C.

2.2 Chemical analyses

Protein was calculated by multiplying the nitrogen content with a factor of 6.25. Dietary fiber and total ash were estimated by the AOAC methods 991.43 and 4.1.10, respectively [14]. Crude lipid was determined by using petroleum ether with a Soxhlet apparatus.

2.3 Diets and animals

According to the formulations of the AIN93M diet [15] with slight modifications (Table 1), two hypercholesterolemic diets, namely cellulose and insoluble FRF diets, were prepared by adding cholesterol (1% w/w) into the diets to induce an alimentary hypercholesterolemia in hamsters. In the cellulose (ICN Nutritional Biochemicals, Cleveland, OH, USA) and insoluble FRF diets, cellulose and insoluble FRF were added as the sole source of fiber, respectively.

Sixteen 6-week-old male Golden Syrian hamsters, weighing 90.8 ± 8.90 g, were obtained from the National Laboratory Animal Center of Taiwan. After a seven-day acclimation period, the animals were randomly allotted to the two diet groups of eight animals each. The hamsters were housed (in pairs) in stainless steel screen-bottomed cages in an air-conditioned room (24 ± 1°C) with a 12 h light : dark cycle. In the whole experimental period (30 days), diets and water were supplied *ad libitum*, and food intake and body weight were recorded daily. Feces were collected and weighed daily, followed by lyophilization. The dried fecal sample were weighed, ground, and stored at –20°C until analyzed. At the end of the experimental period, animals were sacrificed after fasting for 18 h. After anesthesia by ethyl ether, blood was drawn by cardiac puncture, and serum was prepared for biochemical analysis. Liver and other visceral organs were removed, weighed, and stored at –70°C for analysis.

Table 1. Formulations of the experimental diets

Ingredients[a)]	Cellulose diet	Insoluble FRF diet
Casein	14.0	13.3
Cellulose	5.00	–
Insoluble FRF[b)]	–	5.73
Sucrose	10.0	10.0
Corn starch	61.1	61.1
Soybean oil	4.00	4.00
Choline bitartrate	0.25	0.25
L-Cystine	0.18	0.18
AIN-93M vitamin mix	1.00	1.00
AIN-93M mineral mix	3.50	3.50
Cholesterol	1.00	1.00
Energy (kcal/100 g of diet)	376.4[c)]	376.4[c)]

a) The ingredients are expressed as g/100 g of diet (dry weight). Casein, cellulose, choline bitartate, L-cystine, AIN-93M vitamin mix, AIN-93M mineral mix, and cholesterol were obtained from ICN Nutritional Biochemicals (Cleveland, OH, USA).
b) Protein and ash contents in the insoluble FRF were 12.8 and 0.63 g/100 g of FRF, respectively.
c) The percentages of energy of the carbohydrate, protein, and fat were 75.5, 14.9, and 9.6%, respectively.

2.4 Serum cholesterol and triglycerides

Concentration of serum total cholesterol (No. 402; Sigma Chemical, St. Louis, MO, USA), serum high-density lipoprotein (HDL) cholesterol (No. 352; Sigma Chemical), and serum triglycerides (Merckotest 14354; Merck, Darmstadt, Germany) were determined enzymatically using commercial assay kits. Concentration of serum low-density lipoprotein (LDL) cholesterol was determined using the method as described by Allen *et al.* [16].

2.5 Liver lipids

According to the method of Folch *et al.* [17], liver lipid extract was prepared by extracting 1–2 g liver with a chloroform : methanol mixture (2 : 1 v/v). The concentration of liver cholesterol in the liver lipid extract was determined colorimetrically at 490 nm [18]. The total liver lipids in the liver were then quantified gravimetrically by evaporating the solvent in the liver lipid extract.

2.6 Fecal lipids, moisture, bulking effect, and bile acids

Following the methods as described in Section 2.5, fecal cholesterol and total fecal lipids in dried fecal samples were extracted and quantified in the same way like the liver lipids. For the fecal samples collected over the 30 days of the experiment, fecal moisture content was estimated by drying the sample to a constant weight at 105°C. Fecal bulking effects for cellulose and insoluble FRF diets were calculated as follows: fecal bulking effect = [fecal wet weight with fiber consumption (g) – fecal wet weight with fiber-free diet (g)]/amount of fiber intake (g). According to the method of Beher *et al.* [19], fecal bile acids extracts were prepared by refluxing the fecal samples collected over the last 3 days of the experiment with ethanol at 80°C. The content of fecal bile acid in the bile acid extract was determined as described by Chau *et al.* [6].

2.7 Statistical analysis

The collected experimental data were analyzed by one-way analysis of variance (ANOVA) using the statistically analysis system (SAS). Differences were considered to be significant at $P < 0.05$.

3 Results and discussion

Table 1 shows the composition of the test diets, in which the insoluble fibers, such as cellulose and insoluble FRF, were added at 5% level. In this study, the insoluble FRF separated from the defatted passion fruit seed accounted for 89.0% of the defatted seed by weight, and contained small amount of impurities, such as protein and ash (12.8 and 0.63 g/100 g of FRF, respectively). In the presence of protein residues, the exact amount of added insoluble FRF was 5.73 g/100 g of diet. Our previous findings from the passion fruit seed fibers indicated that the insoluble FRF were mainly composed of cellulose, pectic substances, and hemicellulose [13].

On daily observations, all animals remained healthy and active throughout the experiment. After 30 days of feeding, the food intake (8.13–8.57 g/day), body weight gain (0.86–0.99 g/day), and feed efficiency ratio (0.11–0.12 g body weight gain/g feed consumed) of the hamsters between the cellulose and insoluble FRF groups were compared to each other. There were no significant differences in those parameters among the groups. In this study, the cecal pH values of the hamsters fed the cellulose and insoluble FRF diets were 6.69 and 6.22, respectively, implying that cellulose and insoluble FRF might be slightly fermented in the intestinal tract.

Table 2 presents the effects of insoluble fibers on the serum triglyceride and cholesterol concentrations in hamsters. As compared with the cellulose diet, the consumption of insoluble FRF diet could significantly ($P < 0.05$) reduce the serum triglyceride levels in hamsters by 41.2%. The inclusion of other pomace fibers (*e.g.*, apple and orange peel) in diets could also reduce effectively the serum triglyceride levels in rodents [6, 20]. A reduced plasma triglyceride concentration is beneficial to a lower risk of coronary heart disease [21]. The hypolipidemic effect of dietary fiber might be due to the direct interference and alteration in fat and glucose absorption in the intestine [11].

Compared with the cellulose diet (4.61 mmol/L), the feeding of insoluble FRF diet significantly ($P < 0.05$) reduced the serum total cholesterol level by 19.7% (Table 2). The cholesterol-lowering effect might be due to the interference of the intestinal absorption of cholesterol and bile acids by dietary fiber [22]. Table 2 indicates that the HDL cholesterol levels in hamsters were comparable between the two diet groups (2.93–3.29 mmol/L). The significant reductions in serum total cholesterol as well as serum triglycerides might be mainly associated with the reduced level of the LDL cholesterol fraction in serum, leading to a lesser amount of circulating forms of triglycerides and cholesterol [23].

The HDL : total cholesterol ratio for the cellulose and insoluble FRF groups (0.71 and 0.79, respectively) were comparable (Table 2). The relatively high proportion of HDL cholesterol (79% of the total cholesterol) with the insoluble FRF diet might suggest the anti-atherogenic potential of the insoluble FRF. In general, the HDL : total cholesterol ratio is negatively correlated with the risk of coronary heart disease [24, 25]. Since hamsters are usually used for evaluating cholesterol with their metabolism being similar to that of human beings [26], the cholesterol- and lipid-lowering activities of the insoluble FRF determined with hamsters in this study might be a clue to its potential health benefits in humans.

In Table 3, there are no significant differences in the relative liver weight (5.09–5.21 g), liver total lipids (188–193 mg/g of liver), and liver cholesterol (62.7–65.7 mg/g of liver) between the two diet groups. Although the insoluble FRF diet *versus* cellulose diet could result in lower levels of serum triglycerides and total cholesterol, the chemical ana-

Table 2. Effects of insoluble fibers on serum triglyceride and cholesterol concentrations[a)] in hamsters

Diets	Triglycerides (mmol/L)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL : total cholesterol ratio[a)]
Cellulose	1.70 ± 0.02x	4.61 ± 0.56x	3.29 ± 0.33	0.70 ± 0.11	0.71 ± 0.08
Insoluble FRF	1.00 ± 0.13y	3.70 ± 0.47y	2.93 ± 0.42	0.72 ± 0.06	0.79 ± 0.09

a) Data are expressed as mean ± standard deviation ($n = 8$). Values in the same column with different letters are significantly different (Duncan, $P < 0.05$).

Table 3. Effects of insoluble fibers on liver weight[a)], liver total lipids[a)], and liver cholesterol[a)] in hamsters

Diets	Liver weight (g/100 g of body weight)	Liver total lipids (mg/g of liver)	Liver cholesterol (mg/g of liver)
Cellulose	5.21 ± 0.13	188 ± 0.01	62.7 ± 4.12
Insoluble FRF	5.09 ± 0.10	193 ± 0.01	65.7 ± 5.60

a) Data are expressed as mean ± standard deviation (n = 8).

Table 4. Effects of insoluble fibers on the fecal moisture content[a)], fecal wet weight[a)], fecal dry weight[a)], and fecal bulking effect[a)] in hamsters

Diets	Fecal moisture content (g/100 g of feces)	Fecal wet weight (g/day)	Fecal dry weight (g/day)	Fecal bulking effect
Cellulose	33.3 ± 2.67x	1.95 ± 0.18x	1.30 ± 0.18x	1.88 ± 0.37x
Insoluble FRF	37.4 ± 1.00y	3.16 ± 0.23y	1.98 ± 0.22y	4.60 ± 0.42y

a) Data are expressed as mean ± standard deviation (n = 8). Values in the same column with different letters are significantly different (Duncan, $P < 0.05$).

lyses of liver tissues revealed that no apparent variations in the liver lipids were observed between these diet groups.

The influence of insoluble fibers on the fecal moisture content, fecal wet weight, fecal dry weight, and fecal bulking effect in hamsters is demonstrated in Table 4. The results indicate that the consumption of insoluble FRF relative to the cellulose could significantly ($P < 0.05$) increase the fecal moisture content, fecal wet weight, and fecal dry weight up to 112, 162, and 152%, respectively. From the findings of Shankardass *et al.* [27], the fecal weight varied widely with the type and quantity of dietary fiber being consumed. Table 4 shows that the fecal bulking effect with insoluble FRF diet (4.60) was significantly ($P < 0.05$) higher than that with cellulose diet (1.88). The higher fecal moisture content with insoluble FRF diet *versus* cellulose diet might explain the corresponding higher values in the fecal output and fecal bulking effect. The findings from Mongeau *et al.* [28] reported that the feeding of insoluble fibers prepared from celery, parsnip, and rutabaga could also result in an increase in fecal weight and bulk.

Table 5 reveals that the insoluble FRF diet could significantly ($P < 0.05$) elevate the levels of fecal total lipids, fecal cholesterol, and fecal bile acids (120, 127, and 183%, respectively) when compared with the cellulose diet. These results demonstrate that the insoluble FRF was more effective than cellulose in the excretion of total lipids, cholesterol, and bile acids *via* feces. Feeding fibers from the other sources, such as wheat bran, oat bran, and cereals, might also increase the excretion of fecal lipids and sterol [7, 10]. Fruda [29] has stated that fiber with high cation-exchange capacity might destabilize, entrap, and disintegrate the micelles, leading to the reduced absorption of lipids and bile acids. The higher cation-exchange capacity of insoluble FRF (42.5 mequiv/kg) *versus* cellulose (22.7 mequiv/kg) [13] might therefore promote a relatively higher fecal excretion of total lipids and bile acids.

Table 5. Effects of insoluble fibers on fecal total lipids[a)], fecal cholesterol[a)], and fecal bile acids[a)] in hamsters

Diets	Fecal total lipids (mg/day)	Fecal cholesterol (mg/day)	Fecal bile acids (μmol/day)
Cellulose	67.4 ± 3.27x	34.7 ± 2.96x	62.9 ± 6.84x
Insoluble FRF	80.8 ± 4.57y	44.0 ± 1.59y	115 ± 11.4y

a) Data are expressed as mean ± standard deviation (n = 8). Values in the same column with different letters are significantly different (Duncan, $P < 0.05$).

In Tables 2, 3, and 5, high correlations were observed between serum triglycerides and fecal total lipids ($r = -0.98$), and between serum total cholesterol and fecal cholesterol ($r = -0.97$). These results demonstrate that the reduction in serum triglyceride and total cholesterol levels (Table 2) as well as the liver cholesterol level (Table 3) might be partially associated with the enhanced excretion of total lipids *via* feces (Table 5). Furthermore, a negative correlation between serum total cholesterol and fecal bile acids ($r = -0.93$) was observed, suggesting that the reduction in serum total cholesterol level (Table 2) was in part attributed to the promoted excretion of fecal bile acids in stool (Table 5). It was postulated that insoluble FRF might have the ability in binding cholesterol and bile acids in the intestinal lumen, decreasing cholesterol absorption, increasing bile acid excretion, elevating cholesterol catabolism to bile acids, and subsequently upregulating the LDL receptor to compensate the decrease of cholesterol. The excretion of these metabolites and the upregulation of LDL receptors hence resulted in the decrease of circulating LDL cholesterol as well as the hypocholesterolemic effect [9, 30].

4 Concluding remarks

The present study demonstrates that the insoluble FRF prepared from the defatted passion fruit seed had cholesterol- and lipid-lowering effects as compared to cellulose. It effectively ($P < 0.05$) decreased the concentrations of serum triglycerides and total cholesterol, but showed no apparent effect on the concentrations of HDL cholesterol and liver cholesterol. The feeding of insoluble FRF significantly increased ($P < 0.05$) the fecal excretion of total lipids, cholesterol, and bile acids. The consumption of insoluble FRF could also increase ($P < 0.05$) the fecal bulk and moisture. Our results implied that the cholesterol- and lipid-lowering effects of insoluble FRF were in part attributed to the enhanced excretion of lipids and bile acids *via* feces. As the fiber-rich passion fruit seed is available in large quantities as a good source of food fiber, our findings suggest that insoluble FRF could be exploited as a promising hypocholesterolemic ingredient. Nevertheless, further work is necessary in humans to confirm its benefits before it can be accepted as fiber-rich functional food.

5 References

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