

Improvement of the Hypocholesterolemic Activities of Two Common Fruit Fibers by Micronization Processing

SHE-CHING WU,[†] SHIUAN-HUEI WU,[‡] AND CHI-FAI CHAU^{*,‡}

[†]Department of Food Science, National Chiayi University, Chiayi 60004, Taiwan, and

[‡]Department of Food Science and Biotechnology, National Chung Hsing University,
250 Kuokuang Road, Taichung 40227, Taiwan

This study investigated and compared the potential hypocholesterolemic activities of different insoluble fibers (IFs) prepared from carambola and orange pomace with or without micronization processing. After micronization, the cation-exchange and water-holding capacities of these pectic polysaccharide-rich IFs were effectively increased (from 140 to 180% and from 260 to 290%, respectively). The abilities of these microsized fruit IFs to lower the concentrations of serum triglyceride (by 15.6–17.8%) and serum total cholesterol (by 15.7–17.0%) were significantly ($p < 0.05$) improved, possibly by means of enhancing the excretion of cholesterol (123–126%) and bile acids (129–133%) in feces. Fecal moisture content was also increased (127–131%) by the consumption of microsized IFs. These results demonstrated that particle size is an important factor in affecting the characteristics and physiological functions of insoluble fibers. The approach of micronization processing might offer the industry an opportunity to improve the physiological functions of food fibers in fiber-rich functional food applications.

KEYWORDS: Insoluble fiber; carambola; orange; micronization; cholesterol; physicochemical property

INTRODUCTION

Many research efforts in the past two decades have revealed that adequate consumption of dietary fibers can help lower blood lipid and cholesterol, improve intestinal health, and reduce the risk of coronary heart disease as well (1–4). The importance of food fibers has therefore led to an increased consumption of fiber-rich products and, hence, encouraged food scientists to search for new and better sources of fibers as food ingredients.

In the fruit juice industry, *Averrhoa carambola* (also known as carambola or starfruit) and *Citrus sinensis* L. cv. Liucheng (Liucheng sweet orange, LSO) are important tropical fruits for juice production. Thousands of tons of pomace are usually produced after the juice extraction process. The carambola and LSO pomace are rich in insoluble fibers (IFs) materials (508 and 502 g kg⁻¹ of pomace dry weight, respectively), which have desirable functional properties as well as pronounced hypolipidemic and hypocholesterolemic effects (5–8). As the carambola and LSO pomace are available in large quantity after juice production, the insoluble fiber materials could be explored as promising sources of food fibers or low-calorie bulk ingredients in fiber-rich functional food applications.

Our recent findings have revealed that reducing the particle sizes of these promising IFs to microscale could effectively enhance their physicochemical properties (9, 10) as well as intestinal health-enhancement abilities (11, 12). It was suggested that the physicochemical properties and physiological effects of different insoluble fiber particulates might be related to their particle sizes. Because the physicochemical properties of dietary

fibers usually provide clues to their potential physiological effects, for example, cholesterol-lowering action (13, 14), it is hence interesting to investigate the effect of micronization or, in other words, particle size reduction on the potential cholesterol-lowering activity of food fibers.

The objective of this study was to investigate and compare the effects of micronization on the lipid- and cholesterol-lowering abilities of the carambola IF (CIF) and LSO IF (LIF) using a hamster model. Relationships among the chemical composition, physicochemical properties, and physiological aspects of these insoluble fibers (unmicronized and micronized) were also interpreted in this study. The potential applications of microsized insoluble fibers in functional food products as well as the use of micrometer technology in food industry will be discussed.

MATERIALS AND METHODS

Preparation of IFs. The carambola and LSO pomace samples were supplied by the CHIA-MEEI Food Industrial Corp. (Taichung County, Taiwan) after the juice extraction process. The pomace samples were dried in an air-oven at 40 °C for 48 h and were finely ground to 0.5 mm in size. According to the method of Chau and Huang (7), CIF and LIF samples were prepared from the pomace using cold distilled water as a solvent.

Micronization Processing. According to the methods of Chau et al. (10) with slight modifications, CIF and LIF samples were first micronized by pulverising the fiber materials through the milling chamber of a jet mill (JM-1, Yenchen, Taipei, Taiwan) using compressed air at ~65 psi. After that, the fiber particulates with their initial average particle sizes of < 30 μm were mixed with distilled water (1:50, w/v) and further micronized with a high-pressure microsizer (Panda 1000, GEA, Parma, Italy) at a pressure of about 11600 psi. The micronized fiber slurry was then freeze-dried and kept in a desiccator until used.

*Author to whom correspondence should be addressed (telephone +886-4-22852420; fax +886-4-22876211; e-mail chaucf@nchu.edu.tw).

Chemical Composition Analyses. The crude protein content was calculated by multiplying the nitrogen content obtained from a CHN-OS rapid element analyzer (Heraeus F002, Hanau, Germany) by a factor of 6.25. According to Association of Official Analytical Chemists (AOAC) methods (15), moisture (method 934.01) and total ash (method 942.05) were determined. The content of dietary fiber (method 985.29) (15) was quantified using a commercial fiber assay kit (Megazyme K-TDFR, Wicklow, Ireland). For the chemical analysis of fiber components, the neutral sugars profiles as well as the contents of cellulose and Klason lignin of various IF samples were determined using the method described by Chau and Huang (7). Allose was used as an internal standard. The released monosaccharides were quantified as alditol acetates using a gas chromatograph (Hitachi G-5000, Tokyo, Japan) fitted with a flame ionization detector. The conditions were as follows: capillary column, Quadrex 007-225 (15 m × 0.53 mm i.d.); oven temperature, initially held at 100 °C for 3 min, then raised to 220 °C at a rate of 4 °C min⁻¹; injector and detector temperatures, 270 °C; gas flow rates, 2.1 mL min⁻¹ (carrier gas, nitrogen) and 500 mL min⁻¹ (air). Uronic acid was determined colorimetrically according to AOAC method 994.13 (15) using D-galacturonic acid monohydrate as reference.

Physicochemical Properties. According to the method of Chau et al. (9), water-holding capacity (WHC, mL g⁻¹) and solubility (%) of various IF samples were determined. Cation-exchange capacities (CECs) of various IF samples were determined according to the method of Ralet et al. (16). Particle size was estimated by a laser particle size analyzer (Analysette 22-Economy, Fritsch, Germany).

Diets and Experimental Design. According to the AIN93 M formulation (17) with slight modifications, four experimental diets, namely, CIF, LIF, micronized CIF, and micronized LIF diets, were prepared with CIF, LIF, micronized CIF, and micronized LIF as the sole insoluble fiber source, respectively. These four isonitrogenous diets were supplemented with cholesterol (2.0 g kg⁻¹ of diet) to induce an alimentary hypercholesterolemia in hamsters. The diet was prepared by mixing sucrose (100 g kg⁻¹), corn starch (619 g kg⁻¹), soybean oil (40 g kg⁻¹), choline bitartrate (2.5 g kg⁻¹), L-cystine (1.8 g kg⁻¹), AIN-93 M vitamin mix (10 g kg⁻¹), AIN-93 M mineral mix (35 g kg⁻¹), casein, and various IF samples. Most ingredients were obtained from ICN Nutritional Biochemicals (Cleveland, OH). In the CIF, micronized CIF, LIF, and micronized LIF samples, there were small amounts of protein (108, 103, 133, and 114 g kg⁻¹ of IF, respectively) and ash (40, 42, 33, and 35 g kg⁻¹ of IF, respectively). Therefore, the amounts of CIF, micronized CIF, LIF, and micronized LIF incorporated into their corresponding diets were adjusted to 59, 59, 60, and 59 g kg⁻¹, respectively; meanwhile, the levels of casein in the CIF, micronized CIF, LIF, and micronized LIF diets were adjusted to 134, 134, 132, and 133 g kg⁻¹, respectively.

Thirty-two male Golden Syrian hamsters (6 weeks old) with average initial weight of 108 ± 10.2 g were obtained from the National Laboratory Animal Centre of Taiwan. The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University. The institutional guideline for the care and use of laboratory animals has been followed. After an acclimation period of 7 days, the animals were divided into eight weight classes of four each. The four diets were then randomly allocated to one of the four animals in each weight class. The weights of animals among each groups were hence comparable to each other. Animals were housed (in pairs) in screen-bottomed, stainless steel cages in a room maintained at 24 ± 1 °C with a 12 h light/dark cycle. In the whole experimental period (30 days), food and water were supplied ad libitum. Food intake and body weight were recorded every 2 days. Feces were collected, weighed, and analyzed for moisture content every 2 days as well. Some of the fecal samples left unused were stored at -20 °C for further use. On daily observations, all animals remained healthy and active throughout the experiment. At the end of the experiment, all animals were sacrificed after fasting for 12 h. Blood was drawn from the animals by cardiac puncture, and serum was prepared for biochemical analysis. Livers were removed, weighed, and kept at -70 °C for analysis.

Serum Cholesterol and Triglyceride. The concentrations of serum total cholesterol (Merckotest 14366, Merck, Darmstadt, Germany), serum high-density lipoprotein (HDL) cholesterol (Merckotest 14210), and serum triglyceride (Merckotest 14354) were determined enzymatically using commercial assay kits. The concentration of serum low-density lipoprotein (LDL) cholesterol was determined as described by Allen, Bristow, and Yu (18).

Table 1. Proximate Composition^a of Different Insoluble Fiber Samples^b

	composition (g kg ⁻¹ of IF)			
	CIF	micronized CIF	LIF	micronized LIF
protein	108 ± 10	103 ± 8	133 ± 12	114 ± 9
ash	40 ± 6	42 ± 5	33 ± 4	35 ± 4
total monomeric sugars	658 ± 55	653 ± 61	722 ± 44	721 ± 58
rhamnose	155 ± 9	149 ± 13	22 ± 3	22 ± 3
fucose	14 ± 5	14 ± 5	tr ^c	tr
arabinose	35 ± 7	34 ± 5	47 ± 3	46 ± 4
xylose	tr	tr	29 ± 1	30 ± 2
mannose	104 ± 8	108 ± 10	25 ± 3	26 ± 3
galactose	12 ± 2	11 ± 2	44 ± 9	43 ± 5
noncellulosic glucose	131 ± 19	136 ± 14	35 ± 5	37 ± 3
cellulosic glucose	26 ± 2	27 ± 2	216 ± 15	218 ± 11
uronic acid	181 ± 16	174 ± 14	304 ± 59	299 ± 38
other nonsugar components ^d	194 ± 16	202 ± 20	113 ± 12	130 ± 11

^aData are expressed as mean ± standard deviation (*n* = 3). ^bThe amounts of insoluble fiber materials prepared from the carambola and LSO pomace were 508 and 502 g kg⁻¹ of pomace dry weight, respectively. ^cTrace (<0.1). ^dNonsugar components refer to different compounds such as polyphenols and Klason lignin.

Liver and Fecal Lipids. According to the methods described by Huang (19), liver lipids extract was prepared by extracting 1–2 g of liver with a chloroform/methanol mixture (2:1, v/v). The concentration of liver cholesterol in the liver lipids extract was determined colorimetrically at 490 nm. The total liver lipids in the liver were then quantified gravimetrically by evaporating off the solvent in the liver lipids extract. Following the same experimental procedures, fecal lipids were extracted from the dried fecal sample (1–2 g) with a chloroform/methanol mixture (2:1 v/v). Fecal cholesterol and total fecal lipids in the fecal lipids extract were quantified in the same ways that liver lipids were analyzed.

Fecal Bile Acids. Following the method of Beher et al. (20), fecal bile acid 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 acids in the bile acid extract was determined spectrophotometrically at 340 nm (8).

Statistical Analysis. Results were analyzed by one-way analysis of variance and Duncan's multiple-range test using Statistical Analysis System (SAS) software (SAS, Cary, NC). Particle size distribution was presented as geometric means and 95% confident intervals, and statistical analyses were performed with Systat 9.0 for Windows (SPSS, Chicago, IL). Differences were considered to be significant at *p* < 0.05.

RESULTS AND DISCUSSION

Table 1 reveals that the protein contents in CIF and LIF were 108 and 133 g kg⁻¹ of IF, respectively, whereas the ash contents in CIF and LIF were 40 and 33 g kg⁻¹ of IF, respectively. After hydrolysis, the total monomeric sugar contents released from the CIF and LIF were 658 and 722 g kg⁻¹ of IF, respectively. Chemical analyses revealed that the major sugar constituents in the CIF were rhamnose, mannose, noncellulosic glucose, mannose, and arabinose (23.6, 19.9, 15.8, and 5.3% of the total sugars, respectively), whereas those in the LIF were cellulosic glucose, arabinose, galactose, and noncellulosic glucose (29.9, 6.5, 6.1, and 4.9%, respectively) in descending order. Moreover, the uronic acid contents in the CIF and LIF were found to be 27.5 and 42.1% of the total sugars, respectively. In agreement with some previous findings (5, 7), these results suggested that CIF was mainly composed of rhamnose-rich pectic substances and hemicellulose, whereas LIF was mainly composed of pectic substances followed by cellulose. Furthermore, the insoluble fiber samples were found to have some nonsugar components

Table 2. Physicochemical Properties^a of Different Insoluble Fiber Samples

treatment	particle size (μm)		cation-exchange capacity (mequiv kg^{-1})		water-holding capacity (mL g^{-1})	
	CIF	LIF	CIF	LIF	CIF	LIF
without micronization	250 (177–326)x	270 (184–348)x	547 \pm 2x	526 \pm 2x	8.5 \pm 0.5x	15.5 \pm 0.6x
after micronization	9.1 (5.3–18.4)y	7.0 (3.6–15.7)y	750 \pm 3y	944 \pm 4y	22.4 \pm 0.9y	44.5 \pm 1.0y

^a Particle sizes are shown as medians and 95% confidence intervals. Cation-exchange and water-holding capacities are expressed as mean \pm standard deviation ($n = 3$). Values in the same column with different letters are significantly different (Duncan, $p < 0.05$).

Table 3. Concentrations of Serum Triglyceride, Serum Total Cholesterol, Serum HDL Cholesterol, and Serum LDL Cholesterol and HDL/Total Cholesterol Ratio of Hamsters Fed Different Diets^a

diet	triglyceride (mg dL^{-1})	total cholesterol (mg dL^{-1})	HDL cholesterol (mg dL^{-1})	HDL/total cholesterol ratio
CIF	118 \pm 17x	159 \pm 23x	114 \pm 15x	0.71 \pm 0.06
micronized CIF	97 \pm 16y	132 \pm 20yz	102 \pm 13xy	0.77 \pm 0.07
LIF	96 \pm 13y	140 \pm 18xy	99 \pm 15xy	0.71 \pm 0.06
micronized LIF	81 \pm 11z	118 \pm 14z	88 \pm 12y	0.75 \pm 0.06

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

such as polyphenols and Klason lignin (21) in different extents (113–194 g kg^{-1} of IF). After the micronization treatment, no significant differences in the proximate composition between CIF and micronized CIF as well as between LIF and micronized LIF were observed (Table 1).

As shown in Table 2, the application of micronization treatment (i.e., jet-milling followed by high-pressure homogenization) dramatically ($p < 0.05$) reduced the median particle sizes of the CIF and LIF samples by 96.4 and 97.4%, respectively, (from 250–270 to 7.0–9.1 μm). The micronization process hence effectively pulverized the fiber particles to different microsized. Table 2 reveals that the process of micronization effectively ($p < 0.05$) increased the CECs of CIF and LIF (by \sim 1.4- and 1.8-fold, respectively) as well as the WHCs of CIF and LIF (by \sim 2.6- and 2.9-fold, respectively). It was speculated that the higher CEC and WHC of LIF versus CIF after the micronization processing might be partly attributed to the increased surface area, exposing more polar groups or binding sites to the surrounding medium and subsequently increasing the CEC and WHC to different extents (9, 10).

After 30 days of feeding, the food intakes (6.60–6.78 g day^{-1}) and body weight gains (0.71–0.78 g day^{-1}) of the hamsters among the four diet groups were comparable to each other. The similar levels of food intakes hence partly explained the comparable weight gains. Furthermore, no apparent variations in the weights of cecal wall (5.8–6.3 g kg^{-1} of body weight), small intestine (11.4–11.9 g kg^{-1} of body weight), and colon plus rectum (9.6–11.5 g kg^{-1} of body weight) were observed among the four diet groups.

Our previous findings have shown that both the unmiconized carambola and LSO IFs (i.e., CIF and LIF, respectively) relative to cellulose could hinder lipid absorption and have pronounced hypolipidemic activities subsequently (6, 8). In Table 3, our results reveal that the administration of LIF was more effective ($p < 0.05$) than that of CIF in lowering serum triglyceride by 18.6%. As compared with the CIF and LIF diets, the feeding of micronized CIF and micronized LIF diets significantly ($p < 0.05$) reduced the serum triglyceride concentrations by 17.8 and 15.6%, respectively. In general, the decrease in serum triglyceride concentration would effectively reduce the risk of coronary heart disease (1, 14). Table 3 demonstrates that the

Table 4. Liver Weight, Liver Total Lipids, and Liver Cholesterol of Hamsters Fed Different Diets^a

diet	liver wt (g kg^{-1} of bw)	liver total lipids (mg g^{-1} of liver)	liver cholesterol (mg g^{-1} of liver)
CIF	42.9 \pm 5.4	148 \pm 14x	46.4 \pm 2.8x
micronized CIF	42.5 \pm 2.8	130 \pm 23xy	42.4 \pm 5.0xy
LIF	41.9 \pm 2.0	138 \pm 15xy	44.5 \pm 3.6xy
micronized LIF	42.7 \pm 5.7	122 \pm 12y	41.1 \pm 4.1y

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

serum total cholesterol of hamsters between the CIF and LIF diet groups were comparable to each other. As compared with the CIF and LIF diets, the serum total cholesterol concentrations of hamsters fed the micronized CIF and micronized LIF diets were also significantly ($p < 0.05$) lowered by 17.0 and 15.7%, respectively. These results demonstrated that the LIF was more effective ($p < 0.05$) than CIF in lowering the serum total cholesterol, and micronization treatment could markedly ($p < 0.05$) improve the cholesterol-lowering activities of these insoluble fiber materials. Some previous findings from other authors (1, 14, 22) have pointed out that the hypolipidemic and hypocholesterolemic abilities of dietary fibers might be related to their chemical composition, sources, and physicochemical properties. It was hence speculated that the enhanced lipid- and cholesterol-lowering abilities of the micronized insoluble fibers were attributable to their markedly improved physicochemical properties at different extents (5, 7).

In Table 3, no apparent differences in the serum HDL cholesterol concentration were observed between the CIF and LIF groups. The results showed that the treatment of micronization did not lead to any significant changes in the serum HDL cholesterol concentration. Furthermore, the LDL cholesterol concentrations among the CIF, micronized CIF, LIF and micronized LIF groups (22.0 \pm 8.6, 12.2 \pm 8.9, 21.6 \pm 8.2, and 11.3 \pm 5.7 mg dL^{-1} , respectively) were also found to be comparable to each other. The amounts of circulating forms of triglyceride and cholesterol (i.e., LDL cholesterol and very low density lipoprotein cholesterol) in serum were generally related to the serum cholesterol and triglyceride concentrations (23). It was therefore speculated that the reduction in the serum cholesterol and triglyceride concentrations upon the consumption of micronized fiber diets might be partly attributed to the slight decrease in the circulating levels of HDL and LDL fractions in the bloodstream. Furthermore, there is a negative correlation between the HDL/total cholesterol ratio and the risk of coronary heart disease (24, 25). As compared with the HDL/total cholesterol ratios for the CIF and LIF groups (0.71), the slightly elevated ratios for the micronized CIF (0.77) and micronized LIF (0.75) groups implied that the antiatherogenic potential of the micronized carambola and LSO IFs might be slightly higher than their unmiconized forms.

No apparent differences in the relative liver weights (41.9–42.9 g kg^{-1} of body weight) among the four diet groups are observed in Table 4. The variations in the weights and appearances

Table 5. Fecal Weight, Fecal Moisture Content, Fecal Total Lipids, Fecal Cholesterol, and Fecal Bile Acids of Hamsters Fed Different Diets^a

diet	fecal dry wt (g day ⁻¹)	fecal moisture content (g kg ⁻¹ of feces)	fecal total lipids (mg day ⁻¹)	fecal cholesterol (mg day ⁻¹)	fecal bile acid (mg day ⁻¹)
CIF	0.70 ± 0.07	212 ± 27x	56.4 ± 6.1	38.9 ± 5.9x	17.5 ± 2.3x
micronized CIF	0.79 ± 0.10	270 ± 38y	62.1 ± 8.5	48.8 ± 6.1yz	23.2 ± 3.5yz
LIF	0.69 ± 0.09	208 ± 31x	61.0 ± 9.0	40.5 ± 6.4xy	20.0 ± 5.3xy
micronized LIF	0.81 ± 0.09	272 ± 23y	67.1 ± 8.9	49.7 ± 5.5z	25.7 ± 3.6z

^aData are expressed as mean ± standard deviation (n = 8). Values in the same column with different letters are significantly different (Duncan, p < 0.05).

of livers in rats were basically related to the levels of their dietary cholesterol and lipids (26). As shown in **Table 4**, there were no apparent differences in the levels of liver total lipids and cholesterol in hamsters between the CIF and LIF groups or between the micronized CIF and micronized LIF groups. However, the micronized LIF group was found to be statistically effective in lowering the liver total lipids and cholesterol levels (-17.6 and -11.4%, respectively) as compared with the CIF group.

Table 5 shows that the fecal dry weights of hamsters among the four diet groups (0.69–0.81 g day⁻¹) were comparable to each other. The fecal moisture contents of hamsters between the CIF and LIF diet groups were also found to be comparable to each other. However, the feeding of micronized CIF and micronized LIF diets effectively (p < 0.05) increased the fecal moisture contents by ~1.3- and 1.3-fold, respectively, compared with the corresponding unmiconized fiber groups. It was inferred that the significant increase of the fecal moisture content might be partly attributable to the highly elevated WHCs of the micronized CIF and LIF (~2.6- and 2.9-fold, respectively, of their initial values) after appropriate micronization treatment (**Table 2**).

The total lipids, cholesterol, and bile acids in the feces of hamsters fed different diets are summarized in **Table 5**. Among the four diet groups, there were no significant differences in the fecal total lipids (56.4–67.1 mg day⁻¹). The daily fecal excretions of cholesterol and bile acid between the CIF and LIF diet groups were comparable to each other. However, the feeding of the micronized CIF and micronized LIF relative to their unmiconized forms could markedly (p < 0.05) increase the levels of fecal cholesterol (126 and 123%, respectively) and fecal bile acid (133 and 129%, respectively). It was revealed that LIF was far more effective (p < 0.05) than CIF in promoting the excretion of cholesterol and bile acids in stool. The results also demonstrated that the consumption of micronized insoluble fibers could further enhance the excretion of lipids, cholesterol, and bile acids via feces. Furda (27) has described that fiber having high CEC might destabilize, entrap, and disintegrate the micelles, resulting in the reduced diffusion and absorption of lipids, cholesterol, and bile acids. The ability of fibers to bind bile acids in the intestinal lumen might prevent the bile acids from reentering into circulation and being eventually lost through excretion (3, 28). It was therefore speculated that the significantly higher CECs (~1.4- and 1.8-fold, respectively) of the micronized CIF and LIF might promote the excretion of total cholesterol and bile acids via feces.

As for the results mentioned above, the particle size reduction by micronization treatment was effective in enhancing the serum lipid- and cholesterol-lowering abilities of the CIF and LIF samples; meanwhile, the efficacy of micronized LIF in lowering serum lipids and cholesterol was higher than that of micronized CIF. It was inferred that the lipid- and cholesterol-lowering activities that were highly enhanced by the consumption of micronized insoluble fibers could be a combination of the improved physiological performances including increased binding of lipids and bile acids in the intestinal lumen, reduced lipids and cholesterol absorption, increased bile acid excretion, elevated cholesterol catabolism to bile acids, and

subsequent up-regulation of the LDL receptor to compensate the loss of cholesterol. Hence, the excretion of these metabolites and the up-regulation of LDL receptors might result in the reduction of circulating LDL cholesterol concentration as well as the enhancement of hypocholesterolemic effect (3, 14, 23, 29, 30). It should be noted that the above-mentioned cholesterol-lowering effects of different fruit IFs might hold true with the fiber content of not less than 50 g kg⁻¹ of daily diet.

Conclusion. The present study demonstrated that the abilities of different micronized fruit fibers (i.e., micronized CIF and micronized LIF) to lower the concentrations of serum triglyceride (by 15.6–17.8%) and serum total cholesterol (by 15.7–17.0%) were significantly (p < 0.05) improved by means of enhancing the excretion of cholesterol (123–126%) and bile acids (129–133%) in feces. Fecal moisture content was also increased by the consumption of these micronized insoluble fibers. Therefore, micronization treatment was effective in increasing the physicochemical properties such as cation-binding activity and water-holding capacity of the CIF and LIF samples as well as enhancing their serum lipid- and cholesterol-lowering abilities. These results demonstrated that particle size is an important factor in affecting the characteristics and physiological functions of insoluble fibers and hence shed light on the potential applications of micrometer technology in the functional food industry.

ABBREVIATIONS USED

IF, insoluble fiber; LSO, Liucheng sweet orange; CIF, carambola IF; LIF, LSO IF; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

LITERATURE CITED

- Marlett, J. A. Dietary fibre and cardiovascular disease. In *Handbook of Dietary Fibre*; Cho, S. S., Dreher, M. L., Eds.; Dekker: New York, 2001; pp 17–30.
- Slavin, J. L. Dietary fiber and colon cancer. In *Handbook of Dietary Fibre*; Cho, S. S., Dreher, M. L., Eds.; Dekker: New York, 2001; pp 31–45.
- Chen, Z. Y.; Jiao, R.; Ma, K. Y. Cholesterol-lowering nutraceuticals and functional foods. *J. Agric. Food Chem.* **2008**, *56*, 8761–8773.
- Erkkilä, A. T.; Lichtenstein, A. H. Fiber and cardiovascular disease risk: how strong is the evidence?. *J. Cardiovasc. Nursing* **2006**, *21*, 3–8.
- Chau, C. F.; Chen, C. H.; Lee, M. H. Characterization and physicochemical properties of some potential fibers derived from *Averrhoa carambola*. *Nahrung/Food* **2004**, *48*, 43–46.
- Chau, C. F.; Chen, C. H.; Wang, Y. T. Effects of a novel pomace fiber on lipid and cholesterol metabolism in the hamster. *Nutr. Res. (N.Y.)* **2004**, *24*, 337–345.
- Chau, C. F.; Huang, Y. L. Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of *Citrus sinensis* L. Cv. Liucheng. *J. Agric. Food Chem.* **2003**, *51*, 2615–2618.
- Chau, C. F.; Huang, Y. L.; Lin, C. Y. Investigation of the cholesterol-lowering action of insoluble fibre derived from the peel of *Citrus sinensis* L. cv. Liucheng. *Food Chem.* **2004**, *87*, 361–366.
- Chau, C. F.; Wen, Y. L.; Wang, Y. T. Effects of micronization on the characteristics and physicochemical properties of insoluble fibres. *J. Sci. Food Agric.* **2006**, *86*, 2380–2386.

- (10) Chau, C. F.; Wen, Y. L.; Wang, Y. T. Improvement of the functionality of a potential fruit insoluble fibre by micron technology. *Int. J. Food Sci. Technol.* **2006**, *41*, 1054–1060.
- (11) Huang, Y. L.; Sheu, F.; Lee, M. H.; Chau, C. F. Effects of particle size reduction of insoluble fibres by micron technology on various caecal and faecal indices. *J. Sci. Food Agric.* **2008**, *88*, 435–441.
- (12) Wu, S. C.; Chien, P. J.; Lee, M. H.; Chau, C. F. Particle size reduction effectively enhances the intestinal health-promotion ability of an orange insoluble fiber in hamsters. *J. Food Sci.* **2007**, *72*, S618–S621.
- (13) Gordon, D. T. Functional properties vs physiological action of total dietary fibre. *Cereal Foods World* **1989**, *34*, 517–525.
- (14) Lairon, D. Dietary fibres and dietary lipids. In *Advanced Dietary Fibre Technology*; McCleary, B. V., Prosky, L., Eds.; Blackwell Science: Oxford, U.K., 2001; pp 177–185.
- (15) Association of Official Analytical Chemists (AOAC). Methods 934.01, 942.05, 985.29, and 994.13. *Official Methods of Analysis*; AOAC: Arlington, VA, 2003.
- (16) Ralet, M. C.; Della Valle, G.; Thibault, J. F. Raw and extruded fiber from pea hulls. Part I: composition and physicochemical properties. *Carbohydr. Polym.* **1993**, *20*, 17–23.
- (17) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939–1951.
- (18) Allen, K. G. D.; Bristow, S. J.; Yu, L. Hypolipidemic effects of modified psyllium preparations. *J. Agric. Food Chem.* **2004**, *52*, 4998–5003.
- (19) Huang, Y. L. Investigations of the hypocholesterolemic and intestinal health promoting effects of the water extracts from *Amomum villosum* seed, *Zizyphus jujuba* fruit, *Salvia plebeia*, and *Vitis thunbergii*. Ph.D. Dissertation, National Chung Hsing University, Taiwan, 2008; pp 68–71.
- (20) Beher, W. T.; Stradnieks, S.; Lin, G. J.; Sanfield, J. Rapid analysis of human fecal bile acids. *Steroids* **1981**, *38*, 281–295.
- (21) Thomas, M.; Crepeau, M. J.; Rumpunen, K.; Thibault, J. F. Dietary fiber and cell-wall polysaccharides in the fruits of Japanese quince (*Chaenomeles japonica*). *Lebensm. Wiss. Technol.* **2000**, *33*, 124–131.
- (22) Leontowicz, M.; Gorinstein, S.; Bartnikowska, E.; Leontowicz, H.; Kulasek, G.; Trakhtenberg, S. Sugar beet pulp and apple pomace dietary fibres improve lipid metabolism in rats fed cholesterol. *Food Chem.* **2001**, *72*, 73–78.
- (23) Uberoi, S. K.; Vadhera, S.; Soni, G. L. Role of dietary fiber from pulses and cereals as hypocholesterolemic and hypolipidemic agent. *J. Food Sci. Technol.* **1992**, *29*, 281–283.
- (24) Barter, P. J.; Rye, K. A. High density lipoproteins and coronary heart disease. *Atherosclerosis* **1996**, *121*, 1–12.
- (25) Malaspina, J. P.; Bussiere, H.; Calve, G. L. The total cholesterol/HDL cholesterol ratio: a suitable atherogenesis index. *Atherosclerosis* **1981**, *40*, 373–375.
- (26) Beynen, A. C.; Lemmens, A. G.; De Bruije, J. J.; Katan, M. B.; Nab Zutphen, L. F. M. Interaction of dietary cholesterol with cholate in rats: effect of serum cholesterol, liver cholesterol and liver function. *Nutr. Rep. Int.* **1986**, *34*, 557–563.
- (27) Furda, I. Interaction of dietary fiber with lipids mechanistic theories and their limitations. In *New Developments in Dietary Fiber*; Furda, I., Brine, C. J., Eds.; Plenum Press: New York, 1990; pp 67–82.
- (28) Normand, F. L.; Ory, R. L.; Mod, R. R. Binding of bile acids and trace minerals by soluble hemicelluloses of rice. *Food Technol.* **1987**, *2*, 86–90.
- (29) Garcia-Diez, F.; Garcia-Mediavilla, V.; Bayon, J. E.; Gonzalez-Gallego, J. Pectin feeding influences fecal bile acid excretion, hepatic bile acid and cholesterol synthesis and serum cholesterol in rats. *J. Nutr.* **1996**, *126*, 1766–1771.
- (30) Turley, S. D.; Dietschy, J. M. Mechanisms of LDL-cholesterol lowering action of psyllium hydrophilic mucilloid in the hamster. *Biochim. Biophys. Acta* **1995**, *1225*, 177–184.

Received for review March 29, 2009. Revised manuscript received April 28, 2009. Accepted April 30, 2009. This study was financially supported by both the National Science Council of the Republic of China and National Chung Hsing University.