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# Particle Size Reduction Effectively Enhances the Cholesterol-Lowering Activities of Carrot Insoluble Fiber and Cellulose

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This study investigated and compared the effects of particle size reduction on the cholesterol-lowering activities of carrot insoluble fiber-rich fraction (IFF) and plant cellulose. Our results demonstrated that micronization treatment effectively pulverized the particle sizes of these insoluble fibers to different microsizes. Feeding the micronized insoluble fibers, particularly the micronized carrot IFF, significantly ($p < 0.05$) improved their abilities in lowering the concentrations of serum triglyceride (18.6−20.0%), serum total cholesterol (15.5−19.5%), and liver lipids (16.7−20.3%) to different extents by means of enhancing ($p < 0.05$) the excretion of lipids (124−131%), cholesterol (120−135%), and bile acids (130−141%) in feces. These results suggested that particle size was one of the crucial factors in affecting the characteristics and physiological functions of insoluble fibers. Therefore, particle size reduction by micronization might offer the industry an opportunity to improve the physiological functions of insoluble fibers, particularly the carrot IFF, in health food applications.

**KEYWORDS: Insoluble fiber; carrot; cellulose; micronization; particle size; cholesterol**

## INTRODUCTION

Extensive research has demonstrated that sufficient consumption of dietary fibers from different sources, such as cereals, fruits, and vegetables, could promote beneficial physiological functions, including blood lipid and cholesterol attenuation, laxation, and reduced risk of cardiovascular diseases (*1*−*5*). The hypocholesterolemic effects of food fibers are generally related to their composition, source, and physicochemical properties (*2, 6*).

Our previous studies have shown that the pomace of *Daucus carota* (carrot) was rich in insoluble fiber-rich fraction (IFF) (563 g $kg^{-1}$ of pomace), which had desirable functional properties, as well as pronounced hypolipidemic and hypocholesterolemic effects (*7, 8*). Because carrot pomace is available in large quantity after juice production, the carrot IFF could be a promising source of food fiber or low calorie bulk ingredient in functional food applications. Our recent findings have also revealed that reducing the particle size of this promising IFF to microscale could effectively enhance its physicochemical properties (*9*) and intestinal health-enhancement ability (*10*). While physicochemical properties of insoluble fibers usually provide clues to their potential physiological effects, i.e., cholesterol-lowering action (*6*), it is hence interesting to investigate the effect of micronization treatment on the potential cholesterol-lowering activity of insoluble fiber.

The present study was to investigate and compare the effects of particle size reduction of the carrot IFF as well as plant cellulose (control) on the absorption and excretion of lipids and cholesterol in hamsters fed hypercholesterolemic diets supplemented with cholesterol (2.0 g $kg^{-1}$ of diet). The relationship between the physicochemical properties and physiological aspects of these insoluble fibers (unmicronized and micronized) was also interpreted in this study. The potential applications of the micronized insoluble fibers in functional food products as well as the use of micrometer technology in food industry will be discussed in this study.

## MATERIALS AND METHODS

**Carrot IFF and Cellulose Samples.** The pomace sample of *D. carota* was supplied by the CHIA-MEEI Food Industrial Corp. (Taichung County, Taiwan) after the juice extraction process. The carrot pomace was dried in an air oven at 40 °C for 48 h and then finely ground to 0.5 mm in size. According to the method of Chau et al. (*7*), IFF was prepared by homogenizing the carrot sample in cold distilled water (pomace/water ratio at 1:10, w/v) using the Osterizer (Sunbeam-Oster, Niles, IL), followed by filtration and rinsing with 70% ethanol. The carrot IFF collected was dried by solvent exchange and air at 30 °C. The plant cellulose (Alphacel 900453) was purchased from ICN Nutritional Biochemicals (Cleveland, OH).

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**Micronization Treatment.** According to the methods described by Chau et al. (*10*), the micronization was first carried out by pulverizing the insoluble fiber sample in the milling chamber of a jet mill (JM-1, Yenchen, Taipei, Taiwan) at a rate of about 3 g $min^{-1}$ using compressed air at ~65 psi. Fiber particulates of average particle sizes <30 $\mu$m were obtained. Subsequently, the fiber particulates were mixed with distilled water (1:50, w/v) and further homogenized with a high-pressure microsizer (Panda 1000, GEA, Parma, Italy) at a pressure ~11 600 psi. After passing the microsizer for once, the micronized fiber slurry was freeze-dried and kept in a desiccator until used.

**Chemical Analyzes.** Moisture (method 934.01), total ash (method 942.05), and dietary fiber (method 985.29) were determined by the Association of Official Analytical Chemists (AOAC) methods (*11*). The quantification of dietary fiber was performed with the commercial fiber assay kit (Megazyme K-TDFR, Wicklow, Ireland). Crude protein content was estimated by multiplying the nitrogen content obtained from a CHN-OS.

**Physicochemical Properties.** For the measurement of average particle size, insoluble fiber suspension (50 mL) was prepared by mixing the fiber particulate in distilled water (1:100, w/v) and then added into the dispersing unit of a laser particle size analyzer (Analysette 22-Economy, Fritsch, Germany). After automatic homogenization by ultrasonication, the particle size of the fiber sample was measured. Water-holding capacity (WHC, mL $g^{-1}$) of the fiber sample was determined using the method described by Chau et al. (*12*). According to the method of Ralet et al. (*13*), cation-exchange capacity (CEC, mequiv $kg^{-1}$) of the fiber sample was determined.

**Diets and Experimental Design.** According to the AIN93 M formulation (*14*) with slight modifications, four experimental diets, namely, 'IFF', 'micronized IFF', 'cellulose', and 'micronized cellulose' diets, were prepared with carrot IFF, micronized IFF, cellulose, and micronized cellulose as the sole fiber source, respectively. The diets were supplemented with cholesterol (2.0 g $kg^{-1}$ of diet) to induce an alimentary hypercholesterolemia in hamsters. In this study, the 'cellulose' diet was used as a control diet. It was prepared by mixing casein (140 g $kg^{-1}$), cellulose (50 g $kg^{-1}$), 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 $kg^{-1}$), and cellulose (50.0 g $kg^{-1}$). These ingredients were obtained from ICN Nutritional Biochemicals (Cleveland, OH). For the 'IFF', 'micronized IFF', and 'micronized cellulose' diets, the cellulose was replaced by the carrot IFF, micronized IFF, and micronized cellulose, respectively. Because small amounts of protein and ash (76.6 and 37.4 g $kg^{-1}$ fiber, respectively) were present in the IFF samples (unmicronized and micronized), the exact amounts of casein and fiber materials incorporated into the 'IFF' and 'micronized IFF' diets were adjusted to 136 and 56.4 g $kg^{-1}$ of diet, respectively.

A total of 32 male Golden Syrian hamsters (6 weeks old) with an average initial weight of 105 ± 10.9 g were obtained from the National Laboratory Animal Center 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. 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*, and food intake and body weight were recorded every 2 days. Feces were collected, weighed, and analyzed for moisture content daily. Some of the fecal samples left unused were stored at −20 °C for further use. 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. Liver was removed, weighed, and kept at −70 °C for analysis.

**Serum Cholesterol and Triglyceride.** 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 et al. (*15*).

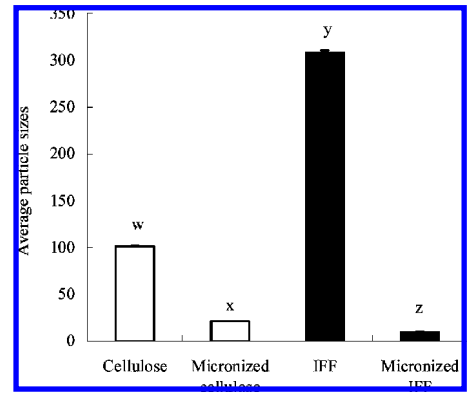


**Figure 1.** Effects of micronization treatment on the average particle sizes ($\mu$m) of cellulose (white bars) and carrot IFF (black bars). Means ($n = 4$) with different letters are significantly different ($p < 0.05$).

**Liver and Fecal Lipids.** According to the method described by Huang (*16*), 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 procedures, fecal cholesterol and total fecal lipids in dried fecal samples were extracted and quantified by the ways that liver lipids were analyzed.

**Fecal Bile Acids.** According to the method of Beher et al. (*17*), 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 (*18*).

**Statistical Analysis.** Experimental data were subjected to one-way analysis of variance and Duncan's multiple-range test using Statistical Analysis System (SAS) software (SAS, Cary, NC). Differences were considered to be significant at $p < 0.05$.

## RESULTS AND DISCUSSION

Chemical analyses revealed that the carrot IFF possessed a considerable amount of insoluble dietary fiber (726 g $kg^{-1}$ of IFF) and also contained a small amount of protein (76.6 g $kg^{-1}$), ash (37.4 g $kg^{-1}$), and other impurities (160 g $kg^{-1}$), such as polyphenols and lignin (*19*). Our previous studies have revealed that the carrot IFF was mainly composed of pectic polysaccharides, hemicellulose, and cellulose (*7*) and also possessed desirable physicochemical properties and physiological functions (*7, 8, 20*). Because the carrot pomace is available in large quantity as a byproduct in juice production, the carrot IFF could be exploited as a promising source of functional food fiber. Furthermore, the insoluble dietary fiber content of the plant cellulose sample was 967 g $kg^{-1}$. This cellulose sample only had a small amount of ash (2.4 ± 0.4 g $kg^{-1}$) and no detectable level of protein and soluble dietary fiber.

**Figure 1** reveals that the application of micronization treatment could effectively ($p < 0.05$) reduce the average particle sizes of the carrot IFF (from 308 to 9.3 $\mu$m) as well as cellulose (from 101 to 20.9 $\mu$m). The particle sizes of carrot IFF and cellulose were dramatically reduced by 97.0 and 79.3%, respectively, of their initial values. These results showed that the micronization treatment (i.e., jet-milling followed by high-pressure homogenization) could effectively pulverize the fiber particles to different microsizes. As shown in **Figures 2** and **3**, the initial WHC and CEC values of cellulose were 4.17 mL $g^{-1}$ and 41.2 mequiv $kg^{-1}$, respectively, while those of the carrot

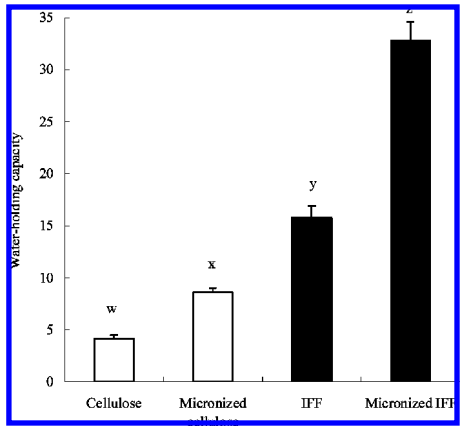


**Figure 2.** Effects of micronization treatment on the water-holding capacities (mL $g^{-1}$) of cellulose (white bars) and carrot IFF (black bars). Means ($n = 4$) with different letters are significantly different ($p < 0.05$).

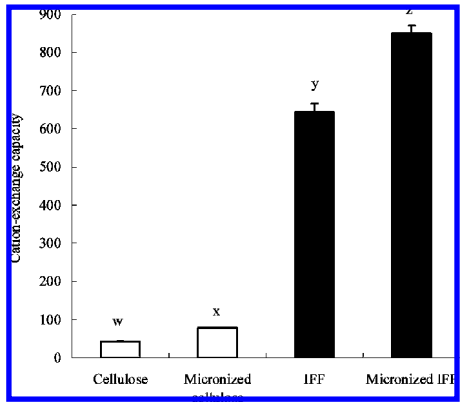


**Figure 3.** Effects of micronization treatment on the cation-exchange capacities (mequiv $kg^{-1}$) of cellulose (white bars) and carrot IFF (black bars). Means ($n = 4$) with different letters are significantly different ($p < 0.05$).

**Table 1.** Food Intake, Body Weight Gain, and Organ Weight of Hamsters Fed Different Diets[a]

| diets | food intake (g $day^{-1}$) | body weight gain (g $day^{-1}$) | cecal wall (g $kg^{-1}$ of body weight) | small intestine (g $kg^{-1}$ of body weight) | colon plus rectum (g $kg^{-1}$ of body weight) |
|---|---|---|---|---|---|
| cellulose | 6.98 ± 0.38 | 0.89 ± 0.18 | 5.6 ± 0.9 | 12.0 ± 1.4 | 10.8 ± 2.1 |
| micronized cellulose | 6.73 ± 0.34 | 0.82 ± 0.14 | 6.0 ± 1.6 | 13.1 ± 2.2 | 12.2 ± 2.8 |
| IFF | 6.95 ± 0.15 | 0.85 ± 0.20 | 5.9 ± 1.3 | 12.0 ± 1.7 | 11.6 ± 1.5 |
| micronized IFF | 6.80 ± 0.35 | 0.81 ± 0.18 | 6.0 ± 1.2 | 12.6 ± 1.6 | 12.6 ± 3.0 |

[a] Data are expressed as mean ± standard derivation ($n = 8$).

IFF were 15.8 mL $g^{-1}$ and 645 mequiv $kg^{-1}$, respectively. The pulverization of cellulose and IFF to microsizes effectively ($p < 0.05$) increased their WHCs (by ~2.1- and 2.1-fold, respectively) as well as their CECs (by ~1.9- and 1.3-fold, respectively). It was inferred that the increased WHCs and CECs of the mincronized insoluble fibers were partly attributed to the structural changes, extended surface area, and also more ion- or water-binding sites being exposed to the surrounding medium after the micronization process (*9, 12*).

**Table 1** shows the composition of the four isonitrogenous diets, in which the fiber content was set at a level of 50 g $kg^{-1}$ of diet. Because the carrot IFF contained a small amount of protein residue (76.6 g $kg^{-1}$ of IFF), the exact amount of IFF added into the two IFF-containing diets was adjusted to 56.4 g $kg^{-1}$ of diet. On daily observations, all animals remained healthy and active throughout the experiment. After 30 days of feeding, the food intakes (6.73−6.98 g $day^{-1}$) and body weight gains (0.81−0.89 g $day^{-1}$) of the hamsters among the four diet groups were comparable to each other. The similar amount of food intakes hence partly explained the comparable weight gains. Moreover, no significant variations in the weights of cecal wall (5.6−6.0 g $kg^{-1}$ of body weight), small intestine (12.0−13.1 g $kg^{-1}$ of body weight), and colon plus rectum (10.8−12.6 g $kg^{-1}$ of body weight) of hamsters among the four diet groups were observed.

**Table 2.** 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]

| diets | serum triglyceride (mg $dL^{-1}$) | serum total cholesterol (mg $dL^{-1}$) | serum HDL cholesterol (mg $dL^{-1}$) | serum LDL cholesterol (mg $dL^{-1}$) | HDL/total cholesterol ratio |
|---|---|---|---|---|---|
| cellulose | 145 ± 14 x | 185 ± 18 x | 121 ± 15 x | 34.6 ± 8.50 x | 0.65 ± 0.03 x |
| micronized cellulose | 116 ± 13 y | 149 ± 12 y | 103 ± 11 y | 22.6 ± 7.23 y | 0.69 ± 0.04 xy |
| IFF | 113 ± 11 y | 155 ± 16 y | 109 ± 12 xy | 23.1 ± 6.47 y | 0.71 ± 0.04 yz |
| micronized IFF | 92 ± 12 z | 131 ± 12 z | 97 ± 11 y | 15.4 ± 5.71 y | 0.74 ± 0.05 z |

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

**Table 2** presents the concentrations of triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, and HDL/total cholesterol ratio in the serum of hamsters among different diet groups. The serum triglyceride concentration of the carrot IFF group was significantly ($p < 0.05$) lower than that of the cellulose group, indicating that the feeding of carrot IFF was more effective than cellulose in lowering the serum triglyceride (−22.1%). Some studies have demonstrated that the administration of pomace fibers derived from various fruits and vegetables were able to reduce the serum triglyceride levels in rodents (*8, 18, 21*). In comparison to the cellulose and carrot IFF diets, the feeding of micronized cellulose and micronized IFF diets effectively ($p < 0.05$) reduced the serum triglyceride concentrations by 20.0 and 18.6%, respectively. It was reported that dietary fiber might hinder lipid absorption directly and subsequently lower the serum triglyceride level and the risk of coronary heart disease (*4, 5*).

As shown in **Table 2**, the serum total cholesterol of the carrot IFF group was 16.2% lower ($p < 0.05$) than that of cellulose group. In comparison to the cellulose and carrot IFF groups, the serum total cholesterol concentrations of the micronized cellulose and micronized IFF groups were significantly ($p < 0.05$) decreased by 19.5 and 15.5%, respectively. The results showed that the carrot IFF was more effective ($p < 0.05$) than cellulose in lowering the serum total cholesterol, and micronization treatment could markedly ($p < 0.05$) improve the cholesterol-lowering activities of these fiber materials. Although a relatively higher level of serum HDL cholesterol was generally expected for a better cardiovascular health, the administration of the micronized cellulose, IFF, and micronized IFF diets in relation to the cellulose diet (121 mg $dL^{-1}$) incurred a significant ($p < 0.05$) decrease in the serum HDL cholesterol concentration by 14.9, 9.9, and 19.8%, respectively. However, in comparison to the HDL/total cholesterol ratio of the cellulose group (0.65), the comparable ratios for the IFF (0.69) and micronized cellulose (0.71) groups as well as the significantly ($p < 0.05$) higher ratio for the micronized IFF group (0.74) were still desirable. It was because there was a negative correlation between the HDL/total cholesterol ratio and the risk of coronary heart disease (*22, 23*).

**Table 3.** Liver Weight, Liver Total Lipids, and Liver Cholesterol of Hamsters Fed Different Diets[a]

| diets | relative liver weight (g $kg^{-1}$ of body weight) | liver total lipids (mg $g^{-1}$ of liver) | liver cholesterol (mg $g^{-1}$ of liver) |
|---|---|---|---|
| cellulose | 45.6 ± 3.2 | 162 ± 13 x | 35.2 ± 4.7 x |
| micronized cellulose | 46.4 ± 8.7 | 135 ± 13 y | 33.2 ± 3.3 xy |
| IFF | 43.0 ± 8.8 | 153 ± 14 x | 29.3 ± 3.2 yz |
| micronized IFF | 42.9 ± 6.2 | 122 ± 15 y | 27.5 ± 3.8 z |

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

**Table 4.** Fecal Weight, Fecal Moisture Content, Fecal Total Lipids, Fecal Cholesterol, and Fecal Bile Acids of Hamsters Fed Different Diets[a]

| diets | fecal dry weight (g $day^{-1}$) | fecal moisture content (g $kg^{-1}$) | fecal total lipids (mg $day^{-1}$) | fecal cholesterol (mg $day^{-1}$) | fecal bile acid (mg $day^{-1}$) |
|---|---|---|---|---|---|
| cellulose | 0.69 ± 0.06 | 168 ± 22 x | 68 ± 7 x | 38.2 ± 4.5 x | 13.8 ± 1.5 x |
| micronized cellulose | 0.73 ± 0.08 | 238 ± 23 yz | 89 ± 11 y | 51.6 ± 5.7 y | 19.4 ± 2.5 y |
| IFF | 0.70 ± 0.09 | 203 ± 23 xy | 87 ± 10 y | 53.9 ± 6.1 y | 18.4 ± 2.2 y |
| micronized IFF | 0.78 ± 0.07 | 266 ± 31 z | 108 ± 9 z | 64.8 ± 5.2 z | 23.9 ± 2.9 z |

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

These results have suggested the anti-atherogenic potential of the carrot IFF and its micronized form.

In **Table 2**, the concentrations of LDL cholesterol with the micronized cellulose, IFF, and micronized IFF diets were significantly ($p < 0.05$) lower than that with the cellulose diet (from −33.2 to −55.5%). The decreased concentrations of LDL-cholesterol fractions in serum leading to a lesser amount of circulating form of cholesterol might partly explain the reduction of serum cholesterol concentration (*24*). It has been reported that the hypolipidemic and hypocholesterolemic abilities of dietary fibers generally depend upon their types, qualities, sources, and physicochemical properties (*4, 5, 21*). It was therefore speculated that the enhancement of the lipid- and cholesterol-lowering abilities of the micronized insoluble fibers was attributable to their markedly improved physicochemical properties at different extent (*9, 12*).

As shown in **Table 3**, no significant differences in the relative liver weights (42.9−46.4 g $kg^{-1}$ body weight) of hamsters among the four diet groups were observed. The variations in the weight and appearance of livers in rats were basically related to the amounts of dietary cholesterol and lipids (*25*). Because the source of fiber was the only variable in the ingredients of the experimental diets, differences in the liver weights were therefore expected to be little. Chemical analyses on liver tissues revealed that there were no significant differences in the liver total lipids (153−162 mg $g^{-1}$ of liver) in hamsters between the cellulose and IFF diet groups, whereas the feeding of carrot IFF versus cellulose was found to be statistically effective in lowering the liver cholesterol level (−16.8%). In comparison to the cellulose and carrot IFF diets, feeding the micronized cellulose and micronized IFF diets resulted in significant ($p < 0.05$) reduction in the liver total lipids (−16.7 and −20.3%, respectively) but not the liver cholesterol.

**Table 4** demonstrates the effects of feeding different insoluble fibers (unmicronized or micronized) on the fecal dry weight and fecal moisture content of hamsters. The fecal weight was generally affected by some dietary factors (e.g., type and quantity of dietary fiber being consumed) (*26*), yet our results demonstrated that the fecal dry weight of hamsters among the four diet groups (0.69−0.78 g $day^{-1}$) were found to be comparable to each other. Upon particle size reduction, the feeding of micronized cellulose and micronized IFF were found to be effective ($p < 0.05$) in increasing the fecal moisture content (up to 142 and 131%) as compared to those of their unmicronized forms. It was inferred that the significant increase of fecal moisture content might be partly attributed to the highly elevated WHCs of the micronized cellulose and IFF (∼2.1- and 2.1-fold, respectively) after micronization treatment (**Figure 2**).

The amounts of total lipids, cholesterol, and bile acids in the feces of hamsters fed different diets were summarized in **Table 4**. Bile acids are the major metabolites of cholesterol. The daily excretion of fecal total lipids, cholesterol, and bile acid of hamsters fed the IFF diet were significantly higher ($p < 0.05$) than those with the cellulose diet (128, 141, and 133%, respectively). In comparison to the cellulose and IFF diets, administration of the micronized cellulose and micronized IFF diets resulted in significant ($p < 0.05$) increases in the levels of fecal total lipids (131 and 124%, respectively), fecal cholesterol (135 and 120%, respectively), and fecal bile acid (141 and 130%, respectively). These results revealed that IFF was far more effective ($p < 0.05$) than cellulose in promoting the excretion of cholesterol and bile acids in stool; meanwhile, the consumption of micronized insoluble fibers could further enhance the excretion of lipids, cholesterol, and bile acids via feces. The ability of dietary fibers to bind bile acids in the intestine might prevent their re-absorption into circulation, leading eventually to the excretion of bile acids in feces (*1, 27*). Furda (*28*) 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. Therefore, the marked elevation in CECs of the micronized cellulose and IFF (∼1.9- and 1.3-fold, respectively) (**Figure 3**) might in part explain the highly increased excretion of total cholesterol and bile acids via feces.

As for the results mentioned above, the particle size reduction by micronization treatment was associated with the enhanced serum lipid- and cholesterol-lowering abilities of cellulose and IFF samples. The efficacy of micronized IFF in lowering serum lipids and cholesterol was higher than that of micronized cellulose. The results indicated that the characteristics and physiological functions of insoluble fibers might be affected by both the particle size reduction and fiber type. Typically, not only does particle size determine the physicochemical property (i.e., water sorption) of fibers, but also other parameters, such as fiber type, chemical structure, and shape, might also play an essential role (*29*). According to the findings from some other studies (*1, 3, 5, 24, 30*), it was speculated that the enhancement in lipid- and cholesterol-lowering activities by the consumption of micronized fibers could be a combination of the improved physiological performances, including decreased transit time, increased binding of lipids and bile acids in the intestinal lumen, reduced absorption of lipids and bile acids, increased bile acid excretion, elevated cholesterol catabolism to bile acids, retarded cholesterol biosynthesis, and subsequently, upregulation of the LDL receptor to compensate for the loss of cholesterol. Hence, the excretion of these metabolites and the upregulation of LDL receptors removed the cholesterol from circulation, leading to the reduction of the circulating LDL cholesterol concentration as well as the enhancement of the hypocholesterolemic effect. It should be noted that the above-mentioned beneficial effects of IFF might hold true when added at a level of no less than 50 g $kg^{-1}$ of diet in the hypercholesterolemic diet containing cholesterol at 2.0 g $kg^{-1}$ of diet.

**Conclusion.** This work therefore demonstrated that the micronization treatment could effectively reduce the particle size of the cellulose and carrot IFF to microsizes. The feeding of the micronized insoluble fibers, particularly the micronized IFF, could significantly ($p < 0.05$) improve their abilities in lowering ($p < 0.05$) the concentrations of serum triglyceride, serum total cholesterol, and liver lipids to different extents by means of enhancing ($p < 0.05$) the excretion of lipids, cholesterol, and bile acids via feces. Fecal moisture content was also increased by the consumption of micronized insoluble fibers. The results suggested that particle size was one of the crucial factors in affecting the characteristics and physiological functions of insoluble fibers. It also sheds light on the potential applications of micrometer technology in the food industry and offers the industries some opportunities to develop new formulations of fiber-rich functional foods.

## ABBREVIATIONS USED

IFF, insoluble fiber fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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