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Physicochemical changes upon micronization process positively improve the intestinal health-enhancement ability of carrot insoluble fibre

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Abstract

The influences of the particle size reduction and physicochemical changes by micron technology on the intestinal health-improvement ability of a promising carrot insoluble fibre was investigated. The feeding of the micronized fibres (10.3–20.9 μm), especially that prepared by the high-pressure micronization, could significantly ($p < 0.05$) improve some caecal and faecal parameters in the intestinal lumen by decreasing caecal ammonia concentration (−25.5%), increasing faecal output (137%) and moisture content (142%), and also reducing the activities of undesired β-D-glucosidase (−42.1%) and β-D-glucuronidase (−68.9%) in faeces. The relationships between the physicochemical properties and physiological functions of the micronized fibres have been discussed. The results also demonstrated that both the particle size and way of treatment were important factors affecting the physicochemical properties and physiological functions of fibres, and the consumption of micronized fibre at 5% level might exert a favourable effect on improving intestinal health.

Keywords: Micronization; Physicochemical properties; Insoluble fibre; Orange peel; Intestinal health; Bacterial enzyme

1. Introduction

Some previous findings have demonstrated that sufficient consumption of dietary fibres could support normal intestinal structure to provide mechanical barrier function, decrease the risk of gastrointestinal disease, and help maintain normal gastrointestinal functions and healthy cardiovascular system (Deng, Liu, He, & Jiang, 2000; Marlett, 2001; Schneeman, 2001; Slavin, 2001). The activities of intestinal mucosal disaccharidases could be used as an index to indicate the functional integrity of intestinal mucosa (Ebihara & Nakamoto, 1998). Moreover, the activities of some faecal bacterial enzymes such as β-D-glucosidase and β-D-glucuronidase in the intestinal lumen and their metabolites formed have also been employed to access the changes in intestinal health, function, and structure (Chau, Sheu, Huang, & Su, 2005; Shiau & Chang, 1983; Takahashi et al., 1995). These enzymes could catalyze a wide range of metabolic transformations and lead to the generation of toxic metabolites and increasing the risk of colon carcinogenesis (Edwards & Rowland, 1992; Shiau & Chang, 1983).

In recent years, there has been a steady increase in carrot juice consumption (Schieber, Stintzing, & Carle, 2001). After juice extraction, thousands of tonnes of carrot pomace are produced in the juice industry, and this agricultural byproduct is generally treated as feed. In a previous study, we reported that carrot pomace was rich in insoluble fibre fraction (IFF) (~56.3 g/100 g of pomace, DW). The IFF prepared from the pomace of carrot (*Daucus carota* L. cv. Heytien) had desirable functional properties, *in vitro* hypoglycemic effect, *in vivo* hypocholesterolemic effect, and *in vivo* intestinal health-promotion effect (Chau, Chen, & Lee, 2004; Chau & Chen, 2006; Hsu, Chien, Chen, & Chau, 2006). As the fibre-rich carrot pomace is available

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in large quantities in juice production, the carrot IFF could be a promising source of food fibre for functional food applications. More research on the function and utilization of this fibre-rich agricultural byproduct would benefit the food industry, juice industry, and the environment.

Micron technology and nanotechnology are emerging technologies which show great potential in nutraceuticals and functional foods for human health improvement (Chen, Weiss, & Shahidi, 2006). The greater surface area per unit mass compared with larger-sized particles of the same chemistry renders tiny particles more biological activity (Oberdörster, Oberdörster, & Oberdörster, 2005; Sanguansri & Augustin, 2006). Our recent findings have revealed that the reduction of particle sizes of this promising IFF to micron-sized fibre powder by different micronizing technologies effectively enhance its physicochemical properties and *in vitro* hypoglycemic potential (Chau, Wang, & Wen, 2007). As the physicochemical properties of fibres usually provide clues to their potential physiological effects (Gordon, 1989), it would be interesting to study the potential physiological functions of the micronized IFF having desirable functionalities.

The present *in vivo* study was carried out in order to evaluate and compare the effects of micronization treatments on the intestinal health-enhancement effects of the carrot IFF. The effects of the micronized fibres on the activities of intestinal mucosal disaccharidases and colonic bacterial enzymes as well as several biochemical parameters in the intestinal tract and faeces were investigated. The relationships among the changes in physicochemical properties and physiological functions of the micronized IFF were also discussed. The understanding of the influences of micronization treatments on the physiological functions of IFF would provide useful insight for exploiting the potential applications of micron technology in fibre-rich food development, food industry, and the environment.

2. Materials and methods

2.1. Preparation of IFF

The pomace sample of *D. carota* was collected from the CHIA-MEEI (Taiwan) Food Industrial Corporation 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. (2004), IFF was prepared by homogenizing the carrot sample in cold distilled water (pomace to water ratio at 1:10, w/v) using the Osterizer (Sunbeam–Oster, Niles, IL, USA), followed by filtration and rinsing with 70% ethanol. The IFF collected was dried by solvent exchange and air at 30 °C.

2.2. Micronization of IFF

The IFF sample was micronized by jet milling and high-pressure micronization according to the methods described by Chau et al. (2007). For the jet-milling, the IFF was pulverized by a single passage of the fibre sample through the milling chamber of a jet-mill (JM-1, Yenchen, Taipei, Taiwan) using a compressed air at ~65 psi. For the high-pressure micronization, the fibre sample with its initial average particle size <30 μm was mixed with distilled water (1:50, w/v), and then micronized with a high-pressure microsizer (Panda 1000, GEA, Parma, Italy) at a pressure about 11,600 psi. After circulating in the microsizer for 10 cycles, the micronized fibre slurry was collected, freeze-dried, and kept in a desiccator until used.

2.3. Chemical analyses

Moisture (method 934.01) and total ash (method 942.05) were determined by the AOAC methods (2000). Crude protein content was estimated by multiplying the nitrogen content obtained from a CHN–OS rapid element analyzer (Heraeus F002, Hanau, Germany) with a factor of 6.25.

2.4. Determination of physicochemical properties

Particle sizes of the fibre samples were estimated by the laser particle size analyzer (Analysette 22-Economy, Fritsch, Germany). The solubility of the IFF sample was determined according to the method as described by Chau et al. (2007). Solubility (%) = [weight (g) of supernatant after dried/weight (g) of IFF] × 100.

2.5. Diets and experimental design

According to the formulation of the AIN93M diet (Reeves, Nielsen, & Fahey, 1993), three test diets, named as control, JM-IFF, and HPM-IFF diets, were prepared by adding the IFF (unmicronized), IFF (micronized by jet-milling), and IFF (micronized by high-pressure micronization) as the sole source of fibre, respectively, thus to evaluate the effects of the fibre samples on the intestinal functions and health. The control diet was prepared by mixing casein (14 g/100 g), sucrose (10 g/100 g), corn starch (67.1 g/100 g), soybean oil (4 g/100 g), choline bitartate (0.25 g/100 g), L-cystine (0.18 g/100 g), AIN-93M vitamin mix (1 g/100 g), AIN-93 M mineral mix (3.5 g/100 g), and unmicronized IFF (5 g/100 g). The JM-IFF and HPM-IFF diets were prepared by replacing the unmicronized IFF with the corresponding micronized fibres. The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University. Twenty-four male Golden Syrian hamsters (6 weeks old) weighing 107 ± 13.0 g were obtained from the National Laboratory Animal Center of Taiwan. After a seven-day acclimation period, animals were divided into eight weight classes of three each. The three diets were then randomly allocated to one of the two animals in each weight class. They were housed (in pairs) in screen-bottomed, stainless steel cage in a room maintained at 24 ± 1 °C, with 12 h light-dark cycles, and had free access to food and water.

The feeding experiment was carried out for 30 days using these three diets. The food intake and body weight were recorded daily. Faeces were collected and weighed daily, and stored at −20 °C until analyzed. At the end of the experiment, animals were sacrificed after fasting for 12 h. After laparotomy, small intestine, caecum, caecal content, and large intestine were collected, weighed, and immediately frozen at −70 °C for further analysis. A portion of faeces was dried by lyophilization to determine the faecal dry weight and faecal moisture content.

2.6. Determination of intestinal disaccharidase activities

The activities of maltase and sucrase in the ileum mucosa were determined according to the method of Dahlqvist (1964). A homogenate of ileum mucosa was prepared with 0.1 M phosphate buffer (pH 7.2) (1:100, w/v) and centrifuged at 3000*g* for 10 min. The activities of maltase and sucrase (μmol of disaccharide hydrolyzed per min per mg of mucosal protein) in the supernatant were determined using the corresponding disaccharide substrates (maltose and sucrose, respectively). Mucosal protein content in the supernatant was determined using commercially available protein assay kit (Cat No 500-0006, Bio-Rad, Hercules, CA, USA).

2.7. Determination of caecal pH and ammonia concentration

According to the method of Shiga et al. (2002), the caecal pH was determined. Following the method of Okuda and Fujii (1986), the caecal content was deproteinized with 95% ethanol and centrifuged at 4025*g* for 5 min. Caecal ammonia content in the supernatant was then determined spectrophotometrically at 630 nm.

2.8. Determination of bacterial enzyme activities in faeces

Using the method of Shiau and Chang (1983), faecal bacterial enzymes in the fresh faecal samples which were collected in the last three days of the feeding experiment were extracted by homogenizing (Glas-Col, Terre Haute, IN, USA) the sample in 0.1 M phosphate buffer (pH 7.2, 1:50, w/v) for 30 min, followed by centrifugation at 1006*g* for 10 min. The supernatant was assayed for β-glucosidase and β-glucuronidase activities. Protein in the supernatant was determined using a commercially available protein assay kit (Cat No 500-0006, Bio-Rad, Hercules, CA, USA).

According to the method of Goldin and Gorbach (1976), β-glucosidase activity (μmol of nitrophenol liberated per min per mg of faecal protein) was determined by using the rate of release of *p*-nitrophenol from 1 mM 4-nitrophenyl β-D-glucopyranoside (N7006, Sigma Chemical Co., St. Louis, MO). The activity of β-glucuronidase (nmol of phenolphthalein produced per min per mg of faecal protein) was determined by the rate of phenolphthalein released from 0.01 M phenolphthalein β-D-glucuronide (P0501, Sigma).

2.9. Statistical analysis

All results were expressed as means ± standard deviation ($n = 8$) except for those of chemical composition and physicochemical properties ($n = 3$). All tests were analyzed by one-way analysis of variance using the Statistical Analysis System (SAS). Values of $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Chemical analyses of carrot IFF

After the carrot juice extraction process, a considerable amount of carrot pomace (~46.4 g/100 g of fresh carrot) was produced, and was collected for the preparation of insoluble fibre sample. Chemical analyses revealed that the carrot pomace was rich in IFF (56.3 g/100 g of pomace, DW), which was the predominant fibre fraction. The IFF only contained a small amount of impurities such as protein (6.94 g/100 g of IFF) and ash (3.65 g/100 g of IFF). A previous study demonstrated that the carrot IFF was mainly composed of pectic polysaccharides, hemicellulose, and cellulose (Chau et al., 2004) and possessed desirable physicochemical properties and different physiological functions (Chau et al., 2004; Chau & Chen, 2006; Hsu et al., 2006). As the carrot pomace is available in large quantity as a byproduct in juice production, it could be exploited as a promising source of functional food fibre.

3.2. Physicochemical changes upon micronization process

Table 1 demonstrates that the applications of micron technologies could reduce the particle sizes of the carrot IFF from its initial average particle size (308 μm) to different micron scales. The jet-milling and high-pressure micronization effectively ($p < 0.05$) reduced the particle size of IFFs by 93.2–96.7% to 20.9 and 10.3 μm, respectively. In the present study, it was revealed that a portion of the insoluble fibre particulates (0.99–5.49% by weight) was dissolved in water after the micronization treatments (Table 1). The increased solubility of the micronized IFFs suggested that there was a redistribution of fibre components from insoluble to soluble fractions by the process of micronization. The elevation in solubility was probably attrib-

Table 1
Effects of micronization on the average particle sizes[a] and solubility[a] of insoluble fibre

Treatments	Average particle size (μm)	Solubility (%)
Without micronization	$308 \pm 2.60x$	tr[b]
After micronization		
Jet-milling	$20.9 \pm 0.42y$	$0.99 \pm 0.04x$
High-pressure micronization	$10.3 \pm 0.22z$	$5.49 \pm 0.30y$

[a] Values in the same column with different letters are significantly different (Duncan, $p < 0.05$).
[b] tr: trace (<0.01).

uted to the tremendously increased surface area and breaking down of structures of the fibre materials during the micronization treatments, resulting in an enhanced solubilization of protein and cell-wall pectic substances (Chau et al., 2007).

3.3. Influences of micronized IFF on the activities of mucosal disaccharidases

All animals remained healthy and active throughout the experiment. Within 30 days of feeding, the food intake (6.32–6.92 g/day) as well as the body weight gain (0.85–0.96 g/day) of hamsters were comparable among the three diet groups. No significant variations in the weights of small intestine (1.17–1.34 g/100 g body weight), caecum (0.59–0.61 g/100 g body weight), and colon plus rectum (1.16–1.31 g/100 g body weight) of hamsters among the three diet groups were observed. These results indicated that there was no apparent influence on the growth of the digestive tract including caecum, small intestine, colon, and rectum after the consumption of micronized carrot fibres. Table 2 presents that the effects of IFFs with or without micronization treatments on the activities of mucosal disaccharidases. There were no significant differences in the activities of maltase and sucrase (6.75–7.29 and 4.32–4.93 μmol of disaccharide hydrolyzed per minute per mg of mucosal protein, respectively) in the intestinal mucosa of hamsters among the three diet groups. The activities of mucosal disaccharidases (i.e. maltase and sucrase) were related to the degree of mucosal injury and intraluminal loss of mucosal cellular material, and could therefore be used as an index to indicate the functional integrity of the intestinal mucosa (Luk, Vaughan, Burke, & Baylin, 1981). In this experiment, the normal growth of animals as well as the comparable activities of these mucosal enzymes suggested that the consumption of micronized IFF at 5% level in diet did not affect the health and integrity of intestinal mucosa.

3.4. Influences of micronized IFF on some caecal parameters

Table 2 summarizes the results of the effects of the IFFs with or without micronization treatments on the caecal pH and ammonia concentration of hamsters. The comparable caecal pH (6.28–6.38) of hamsters among the three diet groups implied that the micronization processes in this study did not alter the fermentability of carrot IFFs. Chemical analyses revealed that the ammonia concentration in the caecal content of hamsters from the HPM-IFF diet group (0.73 μmol of ammonia per gram of caecal content) was significantly ($p < 0.05$) lowered compared to those from the control (−25.5%) and JM-IFF diet groups (−18.0%) (Table 2). Compared to the control, the consumption of JM-IFF diet only led to a slight decrease in the caecal ammonia concentration. The results indicated that micronized IFF prepared by high-pressure micronization was more effective in lowering the amount of harmful ammonia formed in the caecum. A decreased level of ammonia formed in caecum or along the intestinal tract was beneficial for the improvement of intestinal health (Kim, Lee, & Benevenga, 1998). Although no significant changes in the pH values were observed in the caecal content, it was speculated that the remarkable ($p < 0.05$) decrease of caecal ammonia with the HMP-IFF diet was due to the significantly higher water-holding capacity and swelling properties of the micronized carrot insoluble fibre prepared by high-pressure micronization (3.4- to 3.5-fold of the initial value) (Chau et al., 2007), leading to the presence of more water in the caecal content. Consequently, the dilution effect resulted in a decrease of ammonia concentration in the caecal content.

Table 2
Effects of the insoluble fibres with or without micronization treatments on the intestinal enzyme activity, caecal pH, and caecal ammonia concentration

Diets	Maltase[a]	Sucrase[a]	Caecal pH	Caecal ammonia[b,c]
Control	7.29 ± 1.55	4.93 ± 0.62	6.28 ± 0.07	$0.98 \pm 0.18x$
JM-IFF	6.75 ± 1.60	4.41 ± 0.52	6.31 ± 0.10	$0.89 \pm 0.18xy$
HPM-IFF	7.07 ± 1.62	4.32 ± 0.37	6.38 ± 0.26	$0.73 \pm 0.07y$

[a] Specific activities of maltase and sucrase are expressed as μmol of disaccharide hydrolyzed per minute per milligram of mucosal protein.
[b] Caecal ammonia is expressed as μmol of ammonia per gram of caecal content.
[c] Values (means ± standard deviation, $n = 8$) in the same column with different letters indicate significant differences, $p < 0.05$ (Duncan).

3.5. Influences of micronized IFF on some physicochemical parameters in faeces

Table 3 reveals that the faecal dry weight of hamsters from the HPM-IFF group (0.67 g/day) was significantly ($p < 0.05$) higher than the comparable values of the control and JM-IFF groups (134% and 137%, respectively). The results indicated that feeding of micronized IFF prepared by high-pressure micronization could increase the amount of faecal output remarkably. As compared with the relatively mild pulverization process of jet-milling, it was inferred that the intense treatment conditions of high-pressure micronization broke down the fibre structure and exposed more surface area and substrates for the bacterial growth and uses, hence increasing the bacterial mass and faecal dry weight. In general, the faecal dry

Table 3
Effects of the insoluble fibres with or without micronization treatments on the faecal dry weight[a] and faecal moisture content[a]

Diets	Fecal dry weight (g/day)	Fecal moisture content (g/100 g of faeces)
Control	$0.50 \pm 0.08x$	$20.3 \pm 1.79x$
JM-IFF	$0.49 \pm 0.08x$	$24.3 \pm 2.50y$
HPM-IFF	$0.67 \pm 0.03y$	$28.9 \pm 2.32z$

[a] Values (means ± standard deviation, $n = 8$) in the same column with different letters indicate significant differences, $p < 0.05$ (Duncan).

weight might be affected by some dietary factors (e.g. type and quantity of dietary fibre being consumed) (Shankardass et al., 1990), yet these results demonstrated that the processing method (i.e. micronization treatment) was another factor as well.

Table 3 shows that the faecal moisture content with the HMP-IFF diet (28.9 g/100 g of faeces) was significantly ($p < 0.05$) higher than those with the control and JM-IFF diets (142% and 119%, respectively). Our previous findings (Chau et al., 2007) showed that the water holding capacity of the carrot insoluble fibre treated by high-pressure micronization (42.5 ml/g) was significantly higher than that of the unmicronized one (~3.4-fold of the initial value). Moreover, a high correlation ($r = 0.89$) between the water holding capacity and faecal moisture content of the IFFs upon different micronization treatments was also observed. The significantly higher content of faecal moisture with the HMP-IFF diet might hence be explained by the elevated water holding capacity of the micronized IFF processed by high-pressure micronization.

3.6. Influences of micronized IFF on the activities of colonic bacterial enzymes

The effects of the micronized IFFs on the activities of colonic bacterial enzymes (i.e. β-D-glucosidase and β-D-glucuronidase) in faeces are presented in Table 4. As compared with the control group, the feeding of JM-IFF diet led to a slight decrease in the activities of faecal β-D-glucosidase and β-D-glucuronidase (−9.00% and −27.5%, respectively). However, the consumption of micronized IFF prepared by high-pressure micronization could effectively ($p < 0.05$) decrease the activities of β-D-glucosidase and β-D-glucuronidase by 42.1% and 68.9%, respectively. In the large intestine, β-D-glucuronidase and β-D-glucosidase being synthesized by colonic bacteria may hydrolyze the conjugated products of detoxification to liberate toxins, carcinogens, and drugs, and therefore their decreased activities have been associated with lower incidence of colonrectal tumors (Gorbach & Goldin, 1990). Therefore, the highly reduced activities of β-D-glucuronidase and β-D-glucosidase by the consumption of micronized IFFs were desirable for promoting the intestinal health as well as lowering the risk of colon disease. Previous studies have demonstrated that the micronization process might increase the surface area and porosity of fibre materials tremendously and then expose more enzyme-inhibiting substances on the extended fibre surface, consequently leading to the decrease in the activities of various intestinal enzymes (e.g. α-amylase and pancreatic lipase) (Chau et al., 2007). It was therefore speculated that micronization processes, especially the high-pressure micronization, might expose more enzyme-inhibiting substances on the fibre surface and reduce the activity of bacterial enzymes in faeces along the large intestine, hence helped improve the intestinal health.

Table 4
Effects of the insoluble fibres with or without micronization treatments on the activities of faecal bacterial enzymes

Diets	β-D-Glucosidase[a,b]	β-D-Glucuronidase[a,b]
Control	$3.11 \pm 0.82x$	$138 \pm 17.9x$
JM-IFF	$2.83 \pm 0.42xy$ (−9.00%)[c]	$100 \pm 24.6x$ (−27.5%)
HPM-IFF	$1.80 \pm 0.29y$ (−42.1%)	$42.9 \pm 19.2y$ (−68.9%)

[a] Values (means ± standard deviation, $n = 8$) in the same column with different letters indicate significant differences, $p < 0.05$ (Duncan).

[b] Enzyme activities were as described in Section 2.

[c] Data in the parentheses are the percent decreases of the enzymes activities for the JM-IFF and HPM-IFF groups against those of the control group.

4. Conclusions

Reducing the particle sizes of the carrot IFF to micron scales by different micronization treatments (i.e. jet-milling and high-pressure micronization) could result in a redistribution of fibre components from insoluble to soluble fractions and alter their physicochemical properties. The feeding of the micronized IFFs, especially that prepared by the high-pressure micronization, could positively improve some of the caecal and faecal parameters in the intestinal lumen by decreasing caecal ammonia concentration, increasing faecal output and moisture content, and also reducing the activities and harmful metabolites of different bacterial enzymes in faeces. Our results demonstrated that particle size might not be the only factor affecting the characteristics and physiological functions of fibres while the way of processing was also another crucial factor. This study suggests that the incorporation of micronized IFF into diet at 5% level might exert a favourable effect on improving intestinal function and health. It also sheds light on the potential applications of micron technology in the food industry and offers the industries some opportunities to develop new formulations of fibre-rich functional foods.

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