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Different micronization methods significantly improve the functionality of carrot insoluble fibre

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Abstract

This study was to investigate the effects of different micronization methods, including ball milling, jet milling and high-pressure micronization on the characteristics and various functional properties of carrot insoluble fibre-rich fraction (FRF). The results demonstrated that these treatments could effectively ($p < 0.05$) pulverize the fibre particles to different micro-sizes. As particle size decreased, the bulk density of the insoluble FRF was significantly ($p < 0.05$) decreased and a redistribution of fibre components from insoluble to soluble fractions was observed. Furthermore, these treatments, especially the high-pressure micronization, could significantly ($p < 0.05$) increase the physicochemical properties (e.g., water-holding capacity, swelling capacity, oil-holding capacity and cation-exchange capacity), glucose adsorption capacity, α-amylase inhibitory activity and pancreatic lipase inhibitory activity of the insoluble FRF to different extents (from several to 29-fold). Our findings suggested that these micronization treatments would provide an opportunity to improve the functionality of carrot insoluble FRF in food applications.

Keywords: Micronization; Dietary fibre; Particle size; Functional property; Carrot; Pomace

1. Introduction

The beneficial effects of dietary fibre on maintaining normal gastrointestinal function and healthy cardiovascular system and lowering postprandial serum glucose levels (Flourie, 1992; Marlett, 2001; Schneeman, 2001) have encouraged its consumption. The importance of dietary fibre has therefore led to the development of a large and potential market for fibre-rich products and ingredient, and also encouraged food scientists to search for a better source of food fibres. Our previous studies revealed that the insoluble fibre-rich fraction (FRF) prepared from carrot pomace had desirable functional properties, in vitro hypoglycemic effects and in vivo hypolipidemic and hypocholesterolemic effects (Chau, Chen, & Lee, 2004; Hsu, Chien, Chen, & Chau, 2006). As carrot pomace, which is fibre-rich, is available in large quantity in juice production, the carrot insoluble FRF could be a promising source of food fibre or as a low calorie bulk ingredient in functional food applications.

In recent years, the concepts of applying micronization or nanotechnology in food research and development have gained much attention. The reduction of particle sizes of various materials (e.g., metal oxides, metal salts, organic compound and chitosan) by micronization or nanotechnology to micro- or nano-sizes might alter their structures, surface areas and functional properties, and then bring on some new applications in the academic world as well as the industry (Liang, Liu, & Guo, 2002; Masciangioli & Zhang, 2004; Ogawa, Decker, & Mcclements, 2003; Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). Our preliminary studies have revealed that the reduction of particle sizes of dietary fibres to a certain micro-scale might enhance some of their physicochemical properties. A comprehensive understanding of the influences of micronization on the characteristics and different physicochemical properties of dietary fibres would be useful for improving their functionality and potential

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applications. The successful enhancement of the functions of carrot fibre would increase its utilization, and subsequently profit the juice industry, environment and also the development of fibre-rich food.

The objective of this study was to evaluate and compare the effects of different ways of micronization on the particle sizes, characteristics and physicochemical properties of the insoluble FRF prepared from the pomace of carrot (*Daucus carota* L. cv. Heytien). The effects of the micronized insoluble FRF on the in vitro activities of some digestive enzymes were also determined. The potential applications of the micronized insoluble FRF in functional food products, as well as the use of micron technology in the food industry, will be discussed in this study.

2. Materials and methods

2.1. Separation of insoluble FRF

The pomace sample of *D. carota* (orange colour) was collected from the CHIA-MEEI (Taiwan) Food Industrial Corp. 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. Following the method of Hsu et al. (2006), insoluble FRF was prepared by homogenizing the pomace sample in cold distilled water (pomace:water 1:10 w/v) using the Osterizer (Sunbeam-Oster, Niles, IL, USA) at high speed for 1 min. The insoluble FRF was filtered, washed with 70% ethanol and dried by solvent exchange and air at 30 °C.

2.2. Micronization of insoluble FRF

Carrot insoluble FRF was micronized in three different ways using ball mill (PM100, Retsch, Germany), jet mill (JM-1, Yenchen, Taipei, Taiwan) and high-pressure microsizer (Nano150/5, Huayinnano, Beijing, China) separately. For the ball milling, fibre sample and agate balls (3 mm in diameter) in the volume ratio 1:1 (~165 ml each) were added into a 500 ml agate grinding bowl. The fibre sample was then grinded by the agate balls for 5–15 h. For the jet milling, the pulverization was carried out by a single passage of the sample through the milling chamber, using compressed air at ~65 psi. Using a high-pressure microsizer, a fibre sample with its initial average particle size of less than 30 μm was micronized with distilled water at a pressure of about 11,600 psi. After passing through the microsizer once, the micronized mixture was collected and freeze-dried.

2.3. Fibre components and particle size analyses

Moisture (method 934.01), total ash (method 942.05) and soluble dietary fibre (method 985.29) were determined by AOAC methods (2000). Crude protein content was estimated by multiplying the nitrogen content by a factor of 6.25. Before and after micronization, the particle sizes of the fibre samples were estimated by laser particle size analyzer (Analysette 22-Economy, Fritsch, Germany).

2.4. Solubility

The solubility of the insoluble FRF was determined by stirring the sample in distilled water (1:10 w/v) at room temperature for 1 h. After centrifugation (1006*g*) for 10 min, the supernatant and residue were collected, freeze-dried and weighed. Solubility (%) = [weight of supernatant after drying (g)/weight of insoluble FRF (g)] × 100.

2.5. Physicochemical properties

According to the method as described by Chau and Cheung (1999), the cation-exchange capacity (mequiv/kg) of the insoluble FRF was determined. The swelling capacity (ml/g) of the insoluble FRF was determined using the bed volume technique (Ralet, Della Valle, & Thibault, 1993). Water-holding capacity (ml/g) and oil-holding capacity (g/g) of the insoluble FRF were determined by the method of Chau and Huang (2003). The density of vegetable oil being used was 0.85 g/ml. In the determination of bulk density (g/ml), a 10 ml graduated cylinder was gently filled with a known amount of fibre sample. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further decrease in the sample level.

2.6. Determination of glucose adsorption capacity

The glucose adsorption capacity of the insoluble FRF was estimated by stirring 1 g of fibre in 100 ml of glucose solution (100 mmol/l) at 37 °C for 6 h, followed by measuring the final glucose content of the solution (Chau, Huang, & Lee, 2003).

2.7. Determination of inhibitory activity (%) toward α-amylase

In the determination of α-amylase inhibitory activity of the insoluble FRF, 1 g of fibre sample and 4 mg of α-amylase (Cat. No. 100447, ICN Biomedicals, Cleveland, OH) in 40 ml of potato starch solution (4%, w/v) were mixed for 1 h, followed by measuring the final glucose content in the solution (Chau et al., 2003). The inhibitory activity (%) toward amylase was defined as the percent decrease in the glucose production rate over the control (without fibre, 91.0 μmol/h).

2.8. Determination of inhibitory activity (%) toward pancreatic lipase

According to the method of Shimura, Tsuzuki, Kobayashi, and Suzuki (1992) with slight modifications, 0.5 g of fibre sample, 10 ml of olive oil, 50 ml of sodium phosphate buffer (0.1 M, pH 7.2), and 10 ml of pancreatic lipase solution were mixed. The pancreatic lipase solution was

prepared by adding 7.1 mg of pancreatic lipase (L3126, Sigma Chemical Co, St Louis, MO) in 10 ml of sodium phosphate buffer. After incubated in a water bath at 37 °C for 1 h, the test tube was placed in a boiling water bath to cease the reaction. The amount of free fatty acid released was determined by titrating with 0.05 N NaOH. The inhibitory activity (%) toward lipase was defined as the percent decrease in the free fatty acid production rate over the control (without fibre, 39.7 mg of oleic acid/h).

2.9. Statistical analysis

All determinations, which were carried out in triplicates, were analyzed by one-way analysis of variance using the Statistical Analysis System (SAS). Statistical significance was considered at $P< 0.05$.

3. Results and discussion

3.1. Characterization of carrot insoluble FRF

Carrot is an important root vegetable, and usually used for juice production. In many countries, a steady increase of carrot juice consumption has been reported (Schieber, Stintzing, & Carle, 2001). After the juice extraction process, carrot pomace (~46.4 g/100 g of fresh carrot), which was generally disposed of as feed, was collected for fibre preparation in this study. Chemical analyses have revealed that the carrot pomace was rich in insoluble FRF, which was the predominant fibre fraction (56.3 g/100 g of pomace, DW). As the carrot pomace is available in large quantity as a byproduct of juice production, it could be exploited as a good source of food fibre (Chau et al., 2004). There were small amounts of impurities, such as protein and ash (8.38 and 3.72 g/100 g of insoluble FRF, respectively) present in the insoluble FRF. Our previous study has demonstrated that the carrot insoluble FRF was mainly composed of pectic polysaccharides, hemicellulose and cellulose (Chau et al., 2004). In general, insoluble fibre is beneficial to intestinal function as it could help increasing faecal bulk and enhancing intestinal peristalsis (Schneeman, 2001).

3.2. Particle size

Table 1 shows that the initial average particle sizes of the insoluble FRF before micronization was 132 μm. Ball milling (5 and 10 h) could significantly ($p < 0.05$) reduce the particle size to 58.4 (−55.8%) and 12.4 μm (−90.6%), respectively. It showed that the size reduction of the fibre particles was in a time-dependent manner. However, the particle size was only further decreased by a few more percent (−92.6%) even when the milling time was extended to 15 h. Thus, ten hours of grinding would be an appropriate processing time while using ball milling. Jet milling could reduce the particle size to 28.3 μm (−78.6%). Although the final particle size obtained from jet milling was larger than that from ball milling (10 h), the processing time of jet milling (single passage through the jet mill) only took a few seconds and was comparatively more time efficient than ball milling. High-pressure micronization could significantly ($p < 0.05$) reduce the size of the insoluble FRF down to 7.23 μm (−94.5%). These results confirmed that all three treatments could effectively reduce the sizes of the insoluble FRF particles to different micro-sizes. The micronized insoluble FRFs prepared by ball milling (10 h), jet milling and high-pressure micronization were used for all of the other analyses in this study.

Table 1
Effects of micronization on the particle size[a] of the carrot insoluble fibre-rich fraction

Treatments	Particle size (μm)
Without micronization	132 ± 2.10u
After micronization	
Ball milling (5 h)	58.4 ± 1.10v
Ball milling (10 h)	12.4 ± 0.85x
Ball milling (15 h)	9.77 ± 0.06y
Jet milling	28.3 ± 0.33w
High-pressure micronization	7.23 ± 0.36z

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

3.3. Bulk density

As compared with the initial bulk density of the insoluble FRF (0.75 g/ml), Fig. 1 reveals that ball milling (10 h) and jet milling could significantly ($p < 0.05$) decrease the bulk density by 40.0% and 50.7%, respectively. Moreover, the process of high-pressure micronization could even effectively ($p < 0.05$) decrease the bulk density by 96.3%. It was interesting that the bulk density of this micronized insoluble FRF was only ~4% of its initial value. It was observed that the trend of reduction in the bulk density (Fig. 1) paralleled that of particle size (Table 1). The significant reduction of the bulk density might suggest that the porosity as well as the

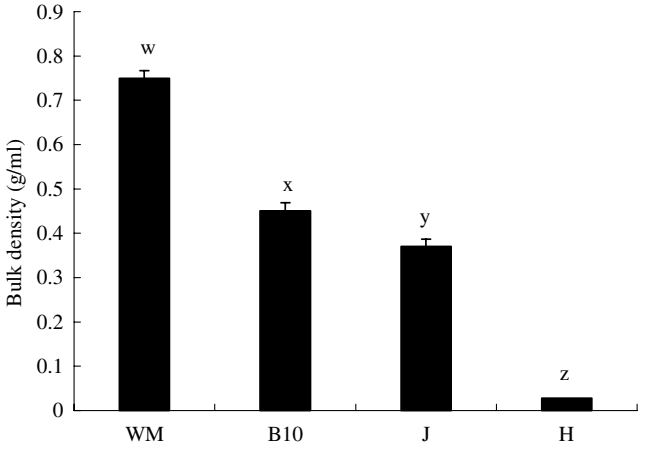

Fig. 1. Effects of micronization on the bulk density (g/ml) of the carrot insoluble fibre-rich fraction. [w–z] Means with different letters are significantly different ($p < 0.05$). WM: without micronization; B10: ball milling for 10 h; J: jet milling; H: high-pressure micronization.

surface area of the fibres could be greatly increased by micronization, especially high-pressure micronization.

3.4. Solubility

Table 2 demonstrates that a portion of the micronized insoluble FRF (2.7–4.4% by weight) dissolved in water after a thorough mixing of it for an hour. The elevated amount of the dissolved fibre fraction indicated that there was a redistribution of fibre components from insoluble to soluble fractions during the micronization process. Chemical analyses indicated that the dissolved fibre fraction formed after ball milling, jet milling and high-pressure micronization contained different levels of protein substances (9.55, 12.4 and 25.6 g/100 g of dissolved insoluble FRF, respectively). Some soluble dietary fibres (25.2 g/100 g of dissolved insoluble FRF) were also found in the dissolved fibre fraction formed after high-pressure micronization. The increased solubility of the insoluble FRF was probably a result of the increased surface area and enhanced solubilization of protein and cell-wall pectic substances by micronization.

3.5. Physicochemical properties

3.5.1. Water-holding and swelling capacities

As shown in Table 2, the water-holding capacities (WHCs) of the micronized insoluble FRFs obtained from ball milling and jet milling (12.6–13.0 ml/g) were comparable to the initial value (12.5 ml/g). The WHCs of fibres isolated from some other fruit and vegetable byproducts (e.g., artichoke, asparagus and cabbage) might vary from 2.80 to 13.2 ml/g (Bao & Chang, 1994; Grigelmo-Miguel & Martin-Belloso, 1999a, 1999b). After treatment by high-pressure micronization, the WHC of the insoluble FRF was significantly ($p < 0.05$) increased by 3.4-fold.

Table 2 shows that the initial swelling capacity of the insoluble FRF (18.0 ml/g) was significantly ($p < 0.05$) increased to 25.7 ml/g (143%) by jet milling; moreover, it could be further increased to 62.2 ml/g (~3.5-fold of the initial value) by the process of high-pressure micronization. The ability of water-binding of dietary fibre was generally related to its structure, density and also the number and nature of its water-binding sites (Chau & Cheung, 1999; Lo, Gordon, & Moore, 1991; Robertson & Eastwood, 1981). It is then speculated that the tremendously reduced particle size (Table 1) and bulk density (Fig. 1) due to high-pressure micronization would expose more surface area, polar groups, uronic acid groups and other water-binding sites to the surrounding water, leading to the significant improvement in the WHC and swelling volume. The high WHC and swelling capacity of the micronized fibres suggested their potential uses as low calorie bulk ingredients in food applications requiring moisture retention.

3.5.2. Oil-holding capacity

Although the process of ball milling did not affect the oil-holding capacity (OHC) of the micronized insoluble FRF in relation to the initial OHC (1.92 g/g), jet milling could significantly ($p < 0.05$) increase the OHC by 168% (Table 2). It should be pointed out that high-pressure micronization could effectively ($p < 0.05$) increase the OHC of the micronized fibre by ~29-fold (to 56.0 g/g) of the initial value. It was inferred that the decrease in bulk density by high-pressure micronization might tremendously increase the porosity and capillary attraction of the fibre, and consequently enhance the physical entrapment of oil and the magnitude of OHC (Chau & Huang, 2003). Although some other authors have reported that a reduction in the particle size of fibres prepared from sugarcane bagasse might lead to a decrease in both the WHC and OHC (Sangnark & Noomhorm, 2003), the changes in the particle sizes and physicochemical properties, upon various micronization treatments (Tables 1 and 2) suggested that particle size of fibres might not be the only factor affecting the physicochemical properties, while the ways of processing were also crucial to the final properties. The high OHC of the micronized insoluble FRFs suggested their potential uses as a fibre-rich ingredient in foodstuffs requiring oil retention, and their potential effects on cholesterol absorption as well.

3.5.3. Cation-exchange capacity

Fig. 2 shows that the three ways of micronization, such as ball milling, jet milling and high-pressure micronization could effectively ($p < 0.05$) increase the cation-exchange capacity (CEC) of the insoluble FRF in an increasing order (111%, 126% and 160%, respectively) as compared to the initial CEC value (624 mequiv/kg). From our previous findings, the carrot insoluble FRF possessed high level of uronic acid (25.9 g/100 g of insoluble FRF) (Chau et al., 2004). Different ways of micronization might expose more surface area, uronic acids or ion binding sites on the insoluble FRF, and consequently increased the CEC to different

Table 2
Effects of micronization on solubility (%)[a], water-holding capacity[a], swelling capacity[a], oil-holding capacity[a] of the carrot insoluble fibre-rich fraction

		After micronization		
	Without micronization	Ball milling (10 h)	Jet milling	High-pressure micronization
Solubility (%)	Tr[b]	2.7 ± 0.0y	1.4 ± 0.1z	4.4 ± 0.0x
Water-holding capacity (ml/g)	12.5 ± 0.06y	13.0 ± 0.15y	12.6 ± 0.13y	42.5 ± 0.05x
Swelling capacity (ml/g)	18.0 ± 0.03z	18.3 ± 0.10z	25.7 ± 0.36y	62.2 ± 0.28x
Oil-holding capacity (g/g)	1.92 ± 0.01z	1.99 ± 0.01z	3.22 ± 0.07y	56.0 ± 0.11x

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

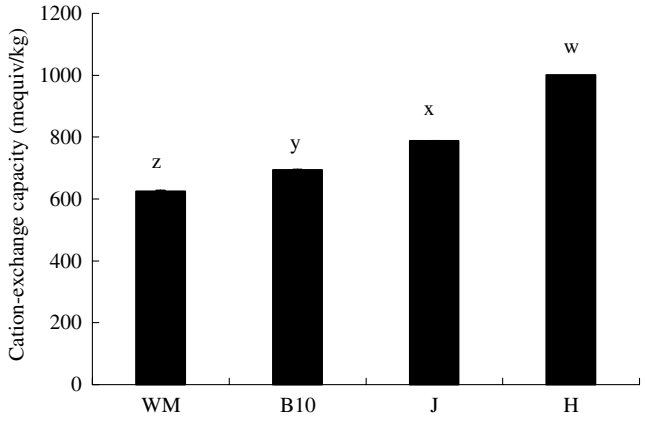

Fig. 2. Effects of micronization on the cation-exchange capacity (mequiv/kg) of the carrot insoluble fibre-rich fraction. $^{w-z}$ Means with different letters are significantly different ($p < 0.05$). WM: without micronization; B10: ball milling for 10 h; J: jet milling; H: high-pressure micronization.

extent. Fibres having high cation-exchange capacity could entrap, destabilize and disintegrate the lipid emulsion, and thus reduce the diffusion and absorption of lipids as well as cholesterol (Furda, 1990; Thibault & Ralet, 2001). Based on the results of different physicochemical properties, it is speculated that high-pressure micronization, which could significantly increase the WHC, swelling property and CEC of the insoluble FRF, might be able to create a concerted effect in reducing the number of intact micelles available, the transit time, and consequently the total time available for cholesterol absorption in the small intestine.

3.6. Glucose adsorption capacity

Fig. 3 reveals that the initial glucose adsorption capacity (GAC) of the insoluble FRF (11.0 mmol/g) in a glucose solution (100 mmol/l) was significantly ($p < 0.05$) increased by ball milling, jet milling and high-pressure micronization (205%, 254% and 584%, respectively). The enhanced GACs of the micronized insoluble FRFs might be due to their

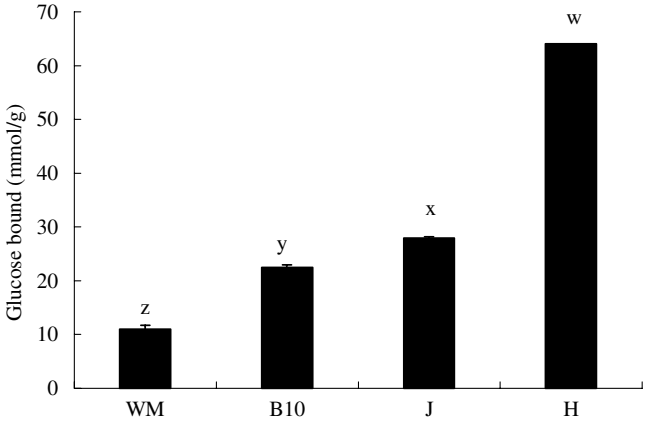

Fig. 3. Effects of micronization on the glucose adsorption capacity (mmol/g) of the carrot insoluble fibre-rich fraction. $^{w-z}$ Means with different letters are significantly different ($p < 0.05$). WM: without micronization; B10: ball milling for 10 h; J: jet milling; H: high-pressure micronization.

increased surface area and porosity for glucose adsorption. Several mechanisms of fibres, including hindering diffusion of glucose, adsorbing glucose, thus reducing the concentration of glucose available in the small intestine, retarding α-amylase action and directly inhibiting the enzyme, could help lower postprandial serum glucose levels (Ou, Kwok, Li, & Fu, 2001). Therefore, it was speculated that the ability to immobilize glucose within the interstices of the micronized fibre particles would help to lower the postprandial glucose concentration available for absorption in the intestinal lumen, and might have potential hypoglycemic effects. It is worth carrying out further investigations on the in vivo hypoglycemic effect of these micronized fibres.

3.7. Inhibitory activity (%) toward pancreatic lipase and α-amylase

Table 3 shows that adding insoluble FRF (without micronization) into a mixture of olive oil and pancreatic lipase solution could reduce the enzyme activity by 9.49%. After particle size reduction, the micronized insoluble FRF exhibited a much stronger inhibitory effect toward pancreatic lipase. Our results demonstrated that ball milling (10 h), jet milling and high-pressure micronization could significantly ($p < 0.05$) increase the lipase inhibitory activity of the insoluble FRF by 247%, 280% and 330%, respectively. It was inferred that micronization, especially the high-pressure micronization, would increase porosity, expose more lipase-inhibiting substances on the fibre surface, enhance capsulation of oil and lipase by fibres, reduce accessibility of lipase to oil, and consequently reduce the lipase activity. These results suggest the potential uses of micronized insoluble FRFs as fibre-rich ingredient in foods, to prevent undesirable changes in foods (e.g., off-flavour), and also to reduce their caloric values produced from edible oil.

Furthermore, insoluble FRF without micronization also had the ability to reduce α-amylase activity by 17.6% (Table 3). The α-amylase inhibitory activity of dietary fibre might be attributed to its ability in embedding starch and enzyme, and even inhibiting the enzyme (Gourgue, Champ, Lozano, & Delort-Laval, 1992; Ou et al., 2001). Table 3 shows that the α-amylase inhibitory activity of micronized insoluble FRF could be significantly ($p < 0.05$) enhanced by ball milling (2.6-fold) and jet milling (2.2-fold). High-pressure micronization could even enhance the lipase inhibitory activity of insoluble FRF by 3.7-fold of its initial value. It is speculated that these micronization treatments effectively increased the porosity and physical interference of the insoluble FRF, causing more amylase-inhibiting substances being exposed on the extended surface and delaying the action of enzyme by embedding the starch and enzyme in the porous fibre network, and then leading to the reduced amylase activity (Bravo, Abia, Goni, & Saura-Calixto, 1995; Chau et al., 2004; Gourgue et al., 1992; Ou et al., 2001). From the results obtained in this in vitro study, the enhanced abilities of the micronized fibres to adsorb glucose and reduce amylase activity implied that they might have

Table 3
Effects of micronization on the inhibitory activity (%)[a] of the carrot fibre-rich fraction toward pancreatic lipase and α-amylase

	Without micronization	After micronization		
		Ball milling (10 h)	Jet milling	High-pressure micronization
Pancreatic lipase inhibitory activity	9.49 ± 0.84z	23.4 ± 0.37y	26.6 ± 0.51x	31.3 ± 0.76w
α-Amylase inhibitory activity	17.6 ± 0.84z	45.1 ± 0.77x	38.3 ± 0.25y	65.1 ± 0.66w

[a] Values in the same row with different letters are significantly different (Duncan, $p < 0.05$).

potential in decreasing the rate of amylolysis and the concentration of postprandial serum glucose.

4. Conclusions

In this study, micronization treatments including ball milling, jet milling and high-pressure micronization could effectively reduce the particle sizes of carrot insoluble FRF to various micro-sizes. After micronization, the bulk density was significantly ($p < 0.05$) decreased and a redistribution of fibre components from insoluble to soluble fractions of the insoluble fibres was observed. Furthermore, these treatments, especially high-pressure micronization, could significantly ($p < 0.05$) increase various physicochemical properties (e.g., WHC, swelling capacity, OHC and CEC), glucose adsorption capacity, α-amylase inhibitory activity and pancreatic lipase inhibitory activity of the insoluble FRF to different extent. The results showed that these micronization treatments would provide an opportunity to improve the functionality of insoluble food fibres, and hence to exploit them as promising low-calorie functional ingredients for fibre enrichment. Micronized FRF could be used in food applications requiring oil and moisture retention, hindering lipid absorption and controlling postprandial serum glucose level. Further investigations of the effects of micronization treatment on the cholesterol- and lipid-lowering activities, promotion of intestinal health and some other physiological functions of different food fibres using animal-feeding experiments are underway. This research would be useful for understanding the potential applications of micronization in food industry.

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