Mechanical Grinding Effects on Health-Related Functional Properties of Dietary Fiber Powder from White Cabbage By-products

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***Abstract*—Health-beneficial effects of Dietary Fiber (DF) are significantly related to its functional properties, which may in turn be altered by fiber structural modification during processing. Although, the potential use of outer leaves of white cabbages (*Brassica oleracea* L. var. *capitata*), residues obtained during industrial processing and selling in the market, as a starting material for production of DF powder has been reported, the effect of processing on the health-related functional properties has not been studied. This work aimed at determining the effect of mechanical grinding on capillary porous structure of DF powder (particle size of less than 450 μm) prepared from white cabbage outer leaves on Glucose Adsorption Capacity (GAC), Glucose Retardation Index (GRI) and Bile acid Retardation Index (BRI). The results showed that DF powder with larger sizes possessed higher GAC, GRI and BRI. The DF product with particle size in the range of 300–450 μm exhibited the highest GAC, GRI and BRI among all samples studied. Scanning electron microscopic (SEM) results showed that applying such an intense mechanical force as grinding to reduce the particle size resulted in rupture of xylem vessels and other tissues. Thus resulted in the collapse of the tube and loss of ability to trap glucose or bile acid into fiber matrix, which hence gave an adverse effect on DF functional properties.**

***Index Terms*—fiber structural, bile acid retardation index, glucose adsorption capacity, glucose retardation index**

I. Introduction

Dietary Fiber (DF) refers to a plant material that is resistant to hydrolysis by human digestive enzymes [1] and can be categorized into Soluble Dietary Fiber (SDF) and Insoluble Dietary Fiber (IDF). SDF consists of noncellulosic polysaccharides (i.e., pectin, gum and mucilages), while IDF consists mainly of cell wall components such as cellulose, lignin and hemicellulose. The physiological and metabolic benefits of DF are generally expressed through the functional properties of the fiber [2]-[4]. For example, DF has the ability to bind bile acids and phospholipids, leading to an increased excretion of bile acids, which results in a lower blood cholesterol level and slower rate of fat absorption into the small intestine [5]. Moreover, DF has the ability to adsorb and entrap glucose molecules within the fiber network, which contribute to the delay of glucose diffusion, leading to postpone absorption and digestion of carbohydrates, thus resulting in lowering postprandial blood glucose [4], [6].

The functional properties of DF are highly dependent on structure and types of DF. During production of DF powder, processing can cause structural alteration, leading as well to changes in the functional properties of the fiber [7], [8]. Grinding the dried material into fine DF powder is one of the most important steps applied after drying. This processing step may affect fiber structure and hence the functional properties. Reduction in particle size can cause varying effects on the fiber properties; different results have been reported for different raw materials. For example, Gupta and Premavalli [9] reported a dramatic increase in SWC with an increase in the particle size of pea peel fiber, while the opposite trend was observed for carrot insoluble fiber [10]. Jongaroontaprangsee *et al.* [11] found that DF powder from lime residues having smaller particle sizes exhibited higher Glucose Retardation Index (GRI) and Bile acid Retardation Index (BRI) values than those of larger particles.

So far, the information on the effect of fiber matrix structure alteration via the process of grinding on functional properties is not clear. An attempt has been made in this work to explain this relationship. Outer leaves of white cabbages (Brassica oleracea L. var. capitata), a by-product from agro-industry and consumer market, was selected as a test material to represent leafy vegetable DF. Moreover, the important health-related functional properties, including Glucose Adsorption Capacity (GAC), GRI and BRI of DF from cabbage outer leaves have not been reported before. The knowledge obtained through this work would provide an understanding of the influence of grinding on the functional properties of the DF product.

II. Materials and Methods

A. DF Powder Preparation

DF powder was prepared as suggested by Tanongkankit *et al.* [12]. In brief, outer leaves of white cabbages (*Brassica oleracea* L. var. *capitata*), which

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were discarded, were obtained from Pakklong Market (Bangkok, Thailand). The leaves were kept at 4 °C until the time of an experiment but no longer than 3 days. Prior to each experiment the leaves were washed under running tap water and drained on a screen to get rid of excess water. The leaves were then steam blanched over boiling water in a closed water bath (Heto, AT 110, Allerod, Denmark) for 1 min. After steam blanching, the leaves were immediately cooled in cold water (4°C). The midrib of each blanched leaf was removed. The remaining leaves were sliced into smaller pieces with the dimensions of 5 × 0.5 cm (length × width).

Approximately 360g of the leaves was placed as a single layer on a sample holder in a vacuum dryer similar to that used by Devahastin *et al.* [13]. Drying was performed at 80°C and at an absolute pressure of 10 kPa as suggested by Tanongkankit *et al.* [12] as the condition that could help maintain the highest amount of phytochemicals in the final product. The leaves were dried to obtain the final moisture content of approximately 0.09 ± 0.01 g/g dry basis [14].

After drying the sample was ground into fine powder using a blender (Waring, model 8011BU, Torrington, CT) at 18,000 rpm for 1 min. The powder was sieved using a sieve analyzer (Retsch, AS200 basic, Haan, Germany). After sieving 4 powder size ranges, i.e., 300−450, 150−300, 75−150 and <75 μm were obtained. The DF powder of each size range was vacuum packed in an aluminum packet and kept at room temperature (~30°C) until further analysis.

B. Determination of Glucose Adsorption Capacity (GAC)

Prior to GAC assays, it was necessary to remove glucose and other free sugars from a sample. This was done as described by Goñi and Martin-Carrón [15]. One g of the DF powder was extracted twice with 20 mL ethanol (85% v/v) for 2 min. After centrifugation at 2500× g for 15 min, the supernatant was discarded and dried at 30 ℃ to remove the ethanol.

The method for the determination of the GAC was that of Ou *et al.* [4] with slight modification. One g of the sugar-free fiber was mixed with 100mL of glucose solution (concentrations between 0−100 mmol/L) and incubated at 37°C for 6h. When the adsorption reached an equilibrium the sample was centrifuged at 4,000× g for 20 min. The glucose content in the supernatant was then determined to estimate the GAC.

Ten μL of the supernatant was injected into Sugar-PakTM 1 (6.5 mm × 300 mm) HPLC column (Waters, Milford, MA). Water was used as a mobile phase and was run at the flow rate of 0.5mL/min. The column was operated at 90°C. Quantification of glucose was carried out based on a glucose standard curve with R^2 of 0.99; the concentration of glucose was calculated from the relative peak area of the standard curve. GAC was calculated as the amount of glucose solution retained by the sample (mmol/g of dry weight) as follows:

$$\text{GAC (mmol/g dry weight)} = \frac{(C_\text{i} - C_\text{S}) \times V_\text{i}}{W_\text{s}} \quad (1)$$

where C_i is the glucose concentration of the original solution (mmol/L), C_s is the glucose concentration when the adsorption reached an equilibrium (mmol/L), W_s is the mass of DF (g dry weight), and V_i is the volume of the glucose solution (L).

C. Determination of Glucose Retardation Index (GRI)

The measurement of GRI was performed using the method described by Chau *et al.* [3] with slight modification. Glucose and other free sugars from a sample was removed as described in Section B. The sugar-free fiber sample (0.2g) was added into 15 mL of glucose solution (50 mmol/L). The mixture was then added into a dialysis membrane with a molecular weight cutoff of 12,000 (P. Intertrade Equipment, 12,000−14,000 MWCO, Seguin, TX). Each membrane was dialyzed against 150 mL of distilled water at 37 °C under constant stirring at 120 rpm in an incubator shaker (New Brunswick Scientific, model G24, Edison, NJ); these procedures were used to simulate the gentle contraction occurring in human intestine. As the *in vivo* time for glucose to enter the intestine was estimated to be under 2 h after food consumption [16], 1 mL of the dialysate was taken at 30, 60 and 120 min time intervals to determine the glucose concentration. A control test was conducted in the same manner but without a fiber sample. Glucose concentration in the dialysate was evaluated via the use of HPLC. The efficiency of DF in lowering the postprandial serum glucose level is expressed in terms of GRI, which can be calculated using the following equation:

$$\text{GRI (\%)} = 100 - \left[\left(\frac{C_\text{gf}}{C_\text{g0}}\right) \times 100\right] \quad (2)$$

where C_gf is total glucose diffused from fiber sample (mmol/L), C_g0 is total glucose diffused from control (mmol/L).

D. Determination of Bile Acid Retardation Index (BRI)

BRI was determined according to the method described by Adiotomre *et al.* [2] with slight modification. Firstly, buffered taurocholate solution was prepared by mixing phosphate buffer at pH 7 with 1 g of sodium azide/L and 15 mmol/L of taurocholic acid (Sigma-Aldrich, Munich, Germany). DF powder sample (0.2g) was added to the buffered taurocholate solution before being filled into a dialysis membrane with a molecular weight cutoff of 12,000. The dialysis bag was placed into 100 mL of phosphate buffer with 1 g sodium azide/L (pH 7); the dialysis was again conducted at 37 °C. The buffered taurocholate solution without the fiber was used as a control. Two mL of the sample was taken at 60 and 120 min to determine the concentration of taurocholic acid in the dialysate.

Taurocholic acid concentration was analyzed by HPLC following the method of Dongowski [17] with some modification. In brief, the diffused taurocholic acid solution was filtered before being injected into Atlantis dC18 5 μL (4.6 mm × 150 mm) HPLC column. A

mixture of 60% methanol in 0.03 M sodium acetate (pH 4.3) (Reagent A) and 90% methanol in 0.03 M sodium acetate (pH 4.3) (Reagent B) was used as a mobile phase at a flow rate of 0.8 mL/min. UV spectrophotometer detector at a wavelength of 205 nm was used to detect taurocholic acid. Quantification of taurocholic acid was carried out based on a standard curve with R^2 of 0.99; the concentration of taurocholic acid was calculated from the relative peak area of the standard curve. BRI was then calculated using the following equation:

$$\text{BRI (\%)} = 100 - \left[\left(\frac{C_{tf}}{C_{t0}}\right) \times 100\right] \quad (3)$$

where C_{tf} is total taurocholic acid diffused from fiber sample (mmol/L), C_{t0} is total taurocholic acid diffused from control (mmol/L).

E. Microstructural Evaluation

The microstructure of the DF powder was examined using a scanning electron microscope (JEOL, JSM-5410LV, Tokyo, Japan). Each powder sample was coated with gold in a sputter coater (Structure Probe, West Chester, PA) before being scanned and photographed at 750× and 1000× magnification.

F. Statistical Analysis

The experiments were designed to be completely random. The data were subject to the analysis of variance (ANOVA) and are presented as mean values with standard deviations. Differences between mean values were established using Duncan's new multiple range tests; the values were considered at a confidence level of 95%. All statistical analyses were performed using SPSS software (version 17) (SPSS Inc., Chicago, IL, USA). All experiments were performed in triplicate unless specified otherwise.

III. Results and Discussion

A. Effect of Grinding on in Vitro Glucose Retardation

A series of different concentrations of glucose (10–100 mmol/L) were used to demonstrate the GAC of the DF powder and the results are given in Fig. 1. The results showed that the DF powder could effectively bind glucose. The ability of the fiber to adsorb glucose was directly proportional to the molar concentration of glucose. By comparing at a similar glucose concentration, the DF powder with the particle size range of 150–300 and 300–450 µm possessed higher GAC than those reported for pea peel fiber (411.2 µmol/g at 50 mmol/L glucose) [9] and insoluble fiber derived from wheat bran (478.3 µmol/g at 50 mmol/L glucose) [4]. The results further illustrated that the powder with the particle size range of 300–450 µm exhibited the highest GAC comparing with the powder having smaller particle sizes. Gupta and Premavalli [9] also observed the higher values of GAC of the fiber powder with a larger particle size. This may be because larger particles could better entrap glucose within the fiber network than smaller particles.

GRI analysis was conducted to evaluate the effect of the DF powder on the delay of glucose absorption in gastrointestinal tract at different times [6]. The movement of glucose across the dialysis membrane gradually increased at every 30-min interval for up to 120 min (Table I). All samples could effectively retard the diffusion of glucose through the dialysis bag as compared with the control.

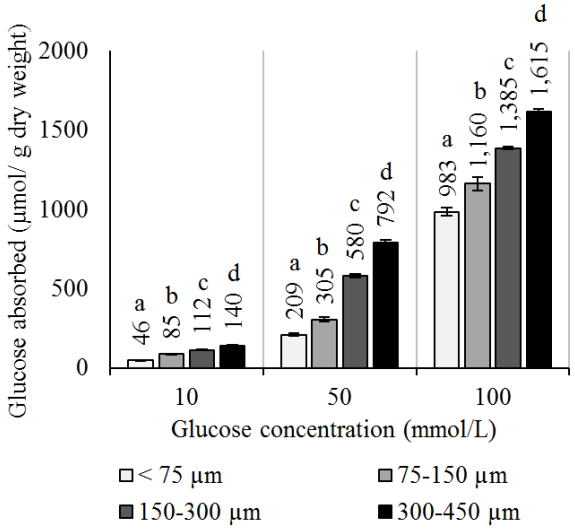

Figure 1. Glucose Adsorption Capacity (GAC) of DF powder at different glucose concentrations (mmol/L). Different letters over the bars belonging to the same glucose concentration indicate that the values are significantly different ($p \leq 0.05$).

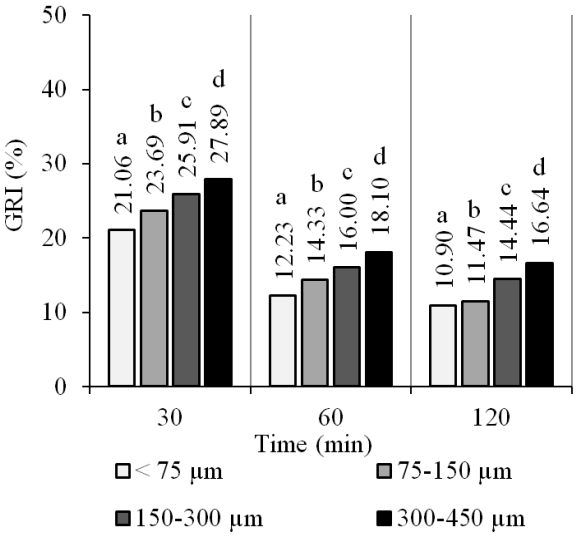

Figure 2. Glucose Retardation Index (GRI) of DF powder having different particle size ranges. Different letters over the bars belonging to the same dialysis time indicate that the values are significantly different ($p \leq 0.05$).

The retardation of glucose diffusion of the DF powder of different particle sizes is expressed by the values of GRI at different times (Fig. 2). The GRI value for the control is 0; the higher the GRI values the higher the ability of the fiber to adsorb glucose within its network [18]. The effect of the particle size on the GRI value was noted to be significant; larger particles better entrapped glucose molecules within the fiber network than the smaller ones leading to the delay of glucose diffusion. The maximum GRI values were obtained after dialysis

for 30 min (21.1–27.9%); these maximum values diminished at prolonged dialysis period. The results of the present study revealed that the DF powder could effectively reduce postprandial hyperglycemia by delaying glucose absorption in the gastrointestinal tract. This may be due to high proportion of IDF; IDF could hinder the diffusion of glucose due to their ability to adsorb and entrap glucose within the fiber network [19]. After 30 to 120 min the rate of retardation of glucose diffusion of the DF powder gradually decreased due to the complete imbibition and saturation of IDF during the first 30 min.

The GRI values (21.1–27.9%) after dialysis for 30 min were noted to be similar to those of many products derived from fruits and vegetables such as orange pulp (25.92%) [20] and lime residue powder (15.96–25.85%) [11]. The values were higher than those of wheat bran (12.6%) [2] and barley (19.3%) [21].

TABLE I. GLUCOSE CONCENTRATION IN DIALYSATE

Particle size (μm)	Glucose concentration in dialysate (mmol/L)		
	Dialysis for 30 min	Dialysis for 60 min	Dialysis for 120 min
300–450	0.962 ±0.012[a]	1.740 ±0.018[a]	2.654 ±0.045[a]
150–300	0.988 ±0.011[b]	1.785 ±0.003[b]	2.724 ±0.004[a]
75–150	1.018 ±0.008[c]	1.820 ±0.005[c]	2.818 ±0.012[b]
<75	1.053 ±0.008[d]	1.865 ±0.030[d]	2.837 ±0.057[b]
Control	1.334 ±0.013[e]	2.125 ±0.018[e]	3.184 ±0.052[c]

Same letters in the same column indicate that values are not significantly different ($p \geq 0.05$).

TABLE II. TAUROCHOLIC ACID CONCENTRATION IN DIALYSATE

Particle size (μm)	Taurocholic acid concentration in dialysate (mmol/L)	
	Dialysis for 60 min	Dialysis for 120 min
300–450	0.206 ± 0.004[a]	0.366 ±0.004[a]
150–300	0.213 ±0.003[b]	0.383 ±0.003[b]
75–150	0.220 ±0.005[c]	0.398 ±0.003[c]
<75	0.230 ±0.001[d]	0.406 ±0.002[d]
Control	0.257 ± 0.003[e]	0.469 ±0.004[e]

Same letters in the same column indicate that values are not significantly different ($p \geq 0.05$).

B. *Effect of Grinding on the in Vitro Bile Acid Retardation*

The cholesterol-lowering effect of DF can be explained by adsorption of bile acids by the fiber. The results showed that the taurocholic concentration diffused from the dialysis bag containing DF powder with larger particle size ranges was significantly lower than those with DF powder with smaller ones and control (Table II). BRI of the DF powder is listed in Fig. 3, where the percentage of taurocholic acid passing into the dialysate is compared with that of the taurocholic control solution.

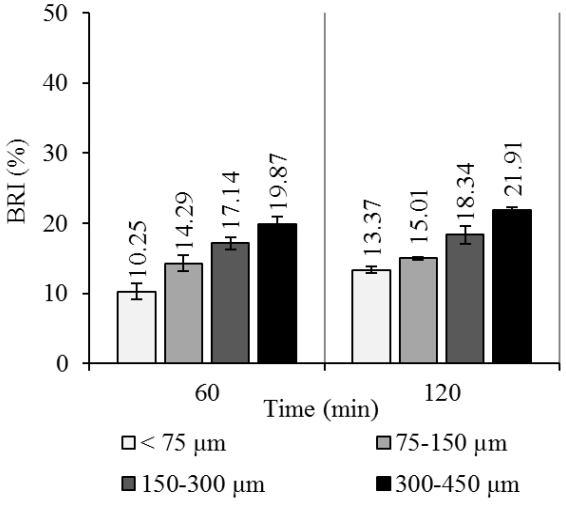

Figure 3. Bile acid Retardation Index (BRI) of DF powder having different particle size ranges. Different letters over the bars belonging to the same dialysis time indicate that the values are significantly different ($p \leq 0.05$).

The results further showed that BRI increased with increased dialysis time and particle size of the powder. The results agree well with those of the previous works. Mongeau and Brassard [22], for example, reported that bile acid (taurocholate) binding capability correlated well with the particle size of neutral detergent fiber from wheat breakfast cereals; larger particle size (in the range of 300–800 μm) exhibited better taurocholate binding.

Particle size exhibited a significant effect on the rate of bile acid diffusion; DF powder with larger particle sizes could retard bile acid diffusion better than those with smaller particle sizes. The powder with the largest particle size (from 300–450 μm) exhibited the highest BRI (21.9%) after dialysis for 120 min. This value is similar to that of lime residue powder having the same particle size (22.2–23.7%) [23], but higher than those of wheat bran (10.5%) and lower than that of pectin (40.2%) [2].

C. *Relationship between Fiber Microstructure and Functional Properties*

To explain the effect of capillary porous structure on the functional properties of the DF powder, SEM images of the powder having different particle sizes were taken. The structure of vascular tissues were mostly noted for the DF powder as cabbages are a dicot plant and their leaves formed by the branched network of veins with the distribution of vascular bundles. Typical xylem vessels appearing as long narrow spiral tubes with thick walls found in cabbage samples are shown in Fig. 4.

Applying such an intense mechanical force as grinding to reduce the particle size clearly resulted in the rupture

of xylem vessels and other tissues (Fig. 5). More intact structure of the vascular tissues was clearly seen in the larger particles, while the damaged xylem vessels were observed in the smaller particles.

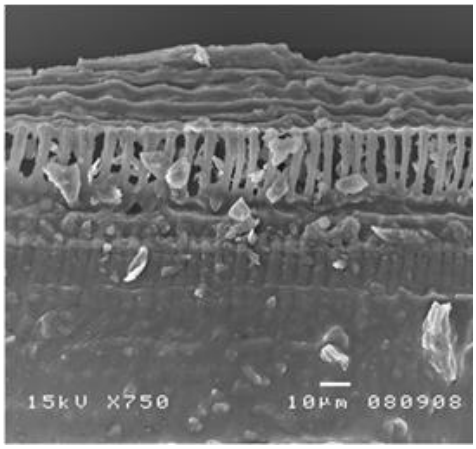

Figure 4. Micrograph (750×) of longitudinal section through xylem vessels of cabbage sample.

Fragments of broken rings from the xylem structure were noted for the samples having the smallest particle size range. In general, xylem composes of primary and secondary walls structured by polysaccharides, including cellulose, hemicellulose and lignin [24] with interconnecting sequences that are disordered and therefore promote functional properties [25]. Auffret *et al.* [26] and Kethireddipalli *et al.* [27] indeed confirmed that the functional properties of the fiber were related to the capillary porous structure formed by polysaccharide chains.

Our results suggested that disintegration of plant tissues by grinding resulted in the loss of the fiber structure, leading to the loss of the ability to bind glucose and bile acid. It is noted that our DF powder possessed high proportion of insoluble fiber, which might play an important role on controlling the diffusion of glucose and bile acid from the dialysis bag. A decrease in GRI and BRI values of the DF powder having smaller particle size was due to the alteration in the integrity of plant cell walls during grinding.

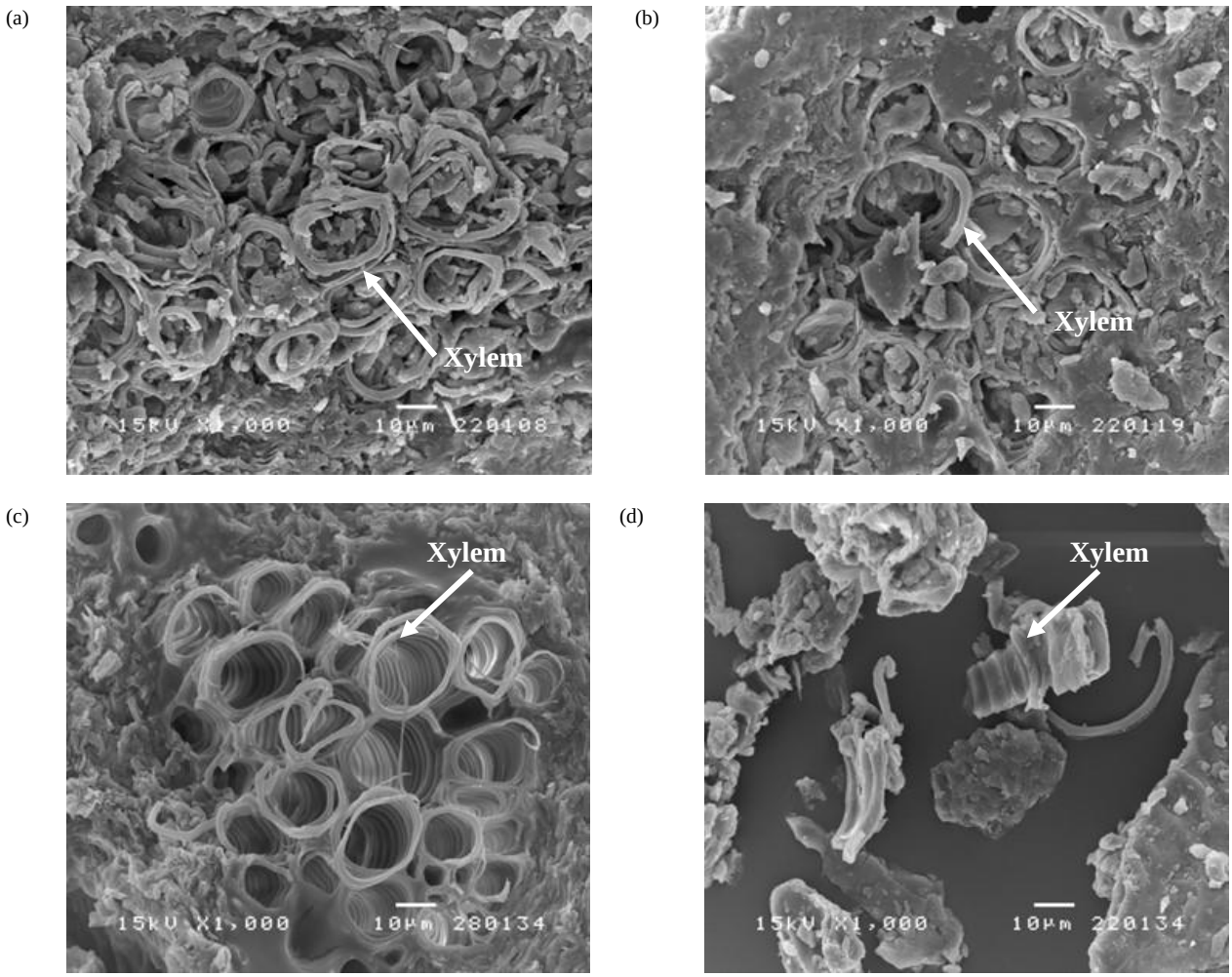

Figure 5. Micrographs (1000×) of damaged vascular tissues as seen in DF powder from cabbage outer leaves having particle size in the range of (a) 300-450 µm; (b) 150-300 µm; (c) 75-150 µm and (d) <75 µm.

IV. Conclusion

The effect of mechanical grinding on selected health-related functional properties of DF powder from cabbage outer leaves was examined. The results showed that grinding had a significant effect on the studied functional properties of the DF powder due to the loss of the capillary porous structure. DF powder with larger sizes possessed higher GAC, GRI and BRI values. DF powder prepared from cabbage outer leaves nevertheless showed excellent performance in relation to health-related functional properties.

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