Article

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Effect of ultrafine grinding on physicochemical and antioxidant properties of dietary fiber from wine grape pomace

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Abstract

Wine grape pomace dietary fiber powders were prepared by superfine grinding, whose effects were investigated on the composition, functional and antioxidant properties of the wine grape pomace dietary fiber products. The results showed that superfine grinding could effectively pulverize the fiber particles to submicron scale. As particle size decrease, the functional properties (water-holding capacity, water-retention capacity, swelling capacity, oil-binding capacity, and nitrite ion absorption capacity) of wine grape pomace dietary fiber were significantly ($p < 0.05$) decreased and a redistribution of fiber components from insoluble to soluble fractions was observed. The antioxidant activities of wine grape pomace and dietary fiber before and after grinding were in terms of DPPH radical scavenging activity, ABTS diammonium salt radical scavenging activity, ferric reducing antioxidant power, and total phenolic content. Compared with dietary fiber before and after grinding, micronized insoluble dietary fiber showed increased ABTS radical scavenging activity, ferric reducing antioxidant power, and total phenolic content yet decreased DPPH radical scavenging activity. Positive correlations were detected between ABTS radical scavenging activity, ferric reducing antioxidant power, and total phenolic content.

Keywords

Grape pomace, superfine grinding, dietary fiber, functional properties, antioxidant properties

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INTRODUCTION

Dietary fiber (DF), defined as 'edible parts of plants or analogous carbohydrate that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine' (AACC, 2001), is abundant in plant products such as fruits, vegetables, and grains. DF has attracted increasing interests in recent years as many studies have revealed that it might be involved in disease preventive and health promotive activities, including attenuation of blood cholesterol and glucose, laxative effect and reduction of risk of colon cancer, heart disease and obesity (Huang et al., 2007; Mclntosh 1993; Zhu et al., 2010). Wine grape pomace (WGP) is primarily composed of seeds, skins and stems, and is commonly used for the extraction of grape seed oils (Deng et al., 2011), and the production of citric acid, methanol, ethanol, and xanthan via fermentation (Couto and Sanroman, 2005). WGP contains multiple types of polyphenols, including 39 types of anthocyanins, hydroxycinnamic acids, catechins, and flavonols (Kammerer et al., 2004). These polyphenols have been claimed to have antioxidant activity (Gonzalez-Paramas et al., 2004) and inhibit low-density lipoprotein oxidation (Yildirim et al., 2005). Furthermore, WGP is a good source of DF. The DF content of

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WGP from grape varieties in Spain, such as Manto Negro, Cencibel, and Airen, has been published (Bravo and Saura-Calixto, 1998; Liobera and Canellask, 2007; Valiente et al., 1995).

In recent years, the possible use of micro- or nano-technology in food research has attracted much attention, and become the focus of research in many countries (Zhu et al., 2010). As particle size decrease, some changes in structure and surface area were made and some new outstanding characteristics were brought. These characteristics (mini-size effect, quantum effect, optical property, magnetic property, chemical and catalytic properties) compared with the conventional particle materials might find some new applications in both academia and industry (Huang et al., 2007; Zhao et al., 2009). Superfine powder is also easier for absorption by the body, which would consequently improve the quality and safety of food products and human health. Fibers are added to cooked meat products to increase the cooking yield owing to their water and fat retention properties. In fried food products, addition of fiber powder reduces lipid retention and increases moisture content (Raghavendra et al., 2004). DFs from different sources differ in chemical composition and physico-chemical properties, and they have been extensively studied for their ability to regulate transit time due to increased stool bulk, and other beneficial properties such as hydration properties like swelling, water-holding capacity (WHC), and water-retention capacity (WRC; Robertson et al., 2000). However, so far the use of this technology in DF processing remains rather limited, probably due to the toughness and polymer nature of DF and inadequate equipment support (Zhu et al., 2010). Although many studies have been conducted on antioxidant DF, yet to date the effect of ultrafine grinding on antioxidant properties of wheat bran DF has been published (Zhu et al., 2010). Since the reduction of particle sizes might cause the release of some antioxidant compounds, it is valuable to find out the antioxidant properties of DF as affected by superfine grinding. Therefore, the aim of this study was to apply the superfine grinding technology for producing DF powder in the submicron range, and to investigate the effects of superfine grinding on the functional and antioxidant properties of insoluble DF. The information obtained from this study will be helpful in the development of value-added products derived from such WGP.

MATERIALS AND METHODS

Materials and chemical

Wine grape (*Cabernet Sauvignon*) pomace was obtained from the Experimental Winery at College of Food Science and Technology, Hebei Normal University of Science and Technology. Cellulase (1 : 1000) was purchased from Beijing Aoboxing Biotechnology Co., Ltd. Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonic acid) (ABTS) diammonium salt, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were purchased from National Standard Samples Center (Beijing, China). All other reagents were of analytical grade.

Preparation of WGP before superfe grinding and insoluble DF

Insoluble dietary fiber (IDF) was prepared from WGP using the procedure described before (Park et al., 2009) with minor modifications. The dried WGP was milled by a laboratory mill (FZ102, Tianjin Taisite Co. Ltd., Tianjin, China) and passed through a 40-mesh screen. The WGP before superfine grinding was obtained for the following step. In order to optimize the yield of components, cold water extraction was used to remove partially soluble polysaccharides, proteins and starches before further treatment. WGP powder (100 g) was sonicated in pH 6.0 water (2000 mL) at 20 °C for 20 min. The cellulase (1 g) was added and the mixture was incubated at 60 °C for 210 min. The enzymes in the suspension were inactivated by heating for 10 min at 100 °C. In this article, 95% ethanol pre-heated to 60 °C was added and the sample was allowed to precipitate at room temperature for 60 min. Finally, the residue was washed with cold water and dried at 50 °C overnight in a vacuum oven to give WGP IDF. The process described above was repeated several times to obtain sufficient WGP IDF for the following step.

Micronization of WGP and IDF

WGP and IDF were micronized by mini-type airflow pulverization system (QLM-80 K, Shangyu City Heli Powder Engineering Co., Ltd, Zhejiang, China). The working frequency was 35 Hz and the working pressure was 70 Mpa. The WGP after superfe grinding and IDF powder obtained was sealed in aluminum foil and kept in a desiccator for further experiments and measurements.

Chemical analysis and particle size measurement of IDF

Total dietary fiber (TDF), IDF, and soluble dietary fiber (SDF) contents were determined as AOAC (2000) methods. Laser diffraction particle size analyzer (LA-920, Horiba Limited, Japan) was employed for the determination of primary particle size distribution.

Functional properties of IDF

Water-holding capacity. WHC is defined by the quantity of water that is bound to the fibers without the application of any external force (except for gravity and atmospheric pressure). Accurately weighed dry sample (1 g) was taken in a graduated test tube, around 30 mL of water was added and it was hydrated for 18 h. The supernatant was removed by passing through a sintered glass crucible (G4) under vacuum. The hydrated residue weight was recorded and it was dried at 105 °C for 2 h to obtain the residual dry weight (Raghavendra et al., 2004).

$$\text{WHC (g/g)} = \frac{\left(\begin{array}{c}\text{(residue hydrated weight}\\ -\text{residue dry weight)}\end{array}\right)}{\text{residue dry weight}}$$

Water-retention capacity. WRC was defined as the quantity of water that remains bound to the hydrated fiber following the application of an external force (pressure or centrifugation). Accurately weighed dry sample (1 g) was taken in a graduated centrifuged tube, around 30 mL of water was added and it was hydrated for 18 h, centrifuged (3000×*g*; 20 min) and the supernatant solution was removed by passing through a sintered glass crucible (G4) under an applied vacuum. The hydrated residue weight was recorded and then sample was dried at 105 °C for 2 h to obtain its dry weight (Raghavendra et al., 2004).

$$\text{WRC (g/g)} = \frac{\left(\begin{array}{c}\text{(residue hydrated weight after}\\ \text{centrifugation} - \text{residue dry weight)}\end{array}\right)}{\text{residue dry weight}}$$

Swelling capacity. Swelling property is defined as the ratio of the volume occupied when the sample is immersed in excess of water after equilibration to the actual weight. Accurately weighed dry sample (0.2 g) was placed in a graduated test tube, around 10 mL of water was added and it was hydrated for 18 h. After 18 h, the final volume attained by fiber was measured (Raghavendra et al., 2004).

$$\text{Swelling capacity (mL/g)} = \frac{\text{volume occupied by sample}}{\text{original sample weight}}$$

Oil-binding capacity. Oil-binding capacity (OBC) was determined by the method of Sangnark and Noomhorm (2003) with slight modifications. A dried sample (5 g) was mixed with peanut oil in a centrifugal tube and left for 1 h at room temperature (25 °C). The mixture was then centrifuged at 1500×*g* for 10 min, the supernatant decanted and the pellet recovered by filtration through a nylon mesh. OBC was expressed as follows:

$$\text{OBC (g/g)} = \frac{\text{(pellet weight} - \text{dry weight)}}{\text{dry weight}}$$

Nitrite ion absorption capacity. A dried sample (1.0 g) was mixed with 100 mL 1 M $NaNO_2$ solution in a 250 mL conical flask. The pH was adjusted to 2.0. The mixture was incubated at 37 °C for 75 min with continuous mild agitation. The residual concentration of nitrite ion was measured. Nitrite ion absorption capacity was expressed as follows:

$$\text{Nitrite ion absorption capacity } (\mu\text{g/g}) = \frac{\left(\begin{array}{c}\text{(nitrite ionbefore absorption}\\ -\text{nitrite ionafter absorption)}\end{array}\right)}{\text{dry weight}}$$

Preparation of antioxidative components of IDF

WGP and WGP DF were separately passed through a 60-mesh screen after grinding by a laboratory mill (FZ102, Tianjin Taisite Co. Ltd, Tianjin, China). The submicron DF powder was directly used in the successive reaction without milling. An amount of 3 g of each sample was then stirred for 1 h in 15 mL *n*-hexane with a magnetic stirrer at ambient temperature and filtered. The residues obtained after filtration were dried and then extracted with 80% methanol (30 mL) by stirring for 2 h with a magnetic stirrer. The mixture obtained was subsequently filtered and filled up to 100 mL with 80% methanol, and then stored in amber colored sealed glass container at 4 °C until further analysis.

Antioxidant properties

Analysis of total phenolic content. The total phenolic content (TPC) of extracts was measured using the Folin–Ciocalteu reagent-based colorimetric assay as described by Singleton et al. (1999). Phenolic content was calculated as gallic acid equivalents and reported as milligram per gram of dry matter. Briefly, 0.5 mL appropriately diluted extract (or gallic acid standard at 0, 50, 100, 150, or 200 ppm) was mixed with 0.5 mL of 2 N Folin–Ciocalteu reagent (Sigma Chemical Co., Saint Louis, MO) and 7.5 mL deionized water and allowed to stand for 10 min at room temperature; then 3 mL of 20% (w/v) Na_2CO_3 was added to the reaction mixture and it was placed in a 40 °C water

bath for 20 min. After the 20 min reaction period, the samples were cooled to room temperature and the absorbance measured at 760 nm (Dong et al., 2011).

DPPH analysis. DPPH radical scavenging capacity of sample was evaluated according to the method of Xu and Chang (2007) with slightly modifications. DPPH radicals have an absorption maximum at 515 nm, which disappears with reduction by an antioxidant compound. The DPPH solution in methanol (6×10^{-5} M) was prepared daily, and 3 mL of this solution was mixed with 100 μL sample solution. The mixture was incubated for 20 min at 37 °C in a water bath, and then the decrease in absorbance at 515 nm was measured (A_E). A blank sample containing 100 μL of methanol in the DPPH solution was prepared daily, and its absorbance was measured (A_B). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula

$$\text{Percentage inhibition} = \left[\frac{(A_E - A_B)}{A_B}\right] \times 100$$

where A_B is the absorbance of the blank sample and A_E the absorbance of the IDF.

ABTS analysis. ABTS was dissolved in deionized water to a 7 mM concentration. ABTS radical cation ($ABTS^+$) was produced by reaction ABTS solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the study, the $ABTS^+$ solution was diluted in deionized water or ethanol to an absorbance of 0.7(±0.02) at 734 nm. An appropriate solvent blank reading was taken (A_B). After the addition of 100 μL of aqueous or ethanolic (according to solubility) sample solutions to 3 mL of $ABTS^+$ solution, the absorbance reading was taken at 30 °C 10 min after initial mixing (A_E). All solution was used on the day of preparation, and all determinations were carried out in triplicate (Dudonne et al., 2009). The percentage of inhibition of $ABTS^+$ was calculated using the following formula

$$\text{Percentage inhibition} = \left[\frac{(A_E - A_B)}{A_B}\right] \times 100$$

where A_B is the absorbance of the blank sample and A_E the absorbance of the IDF.

Ferric reducing antioxidant power assay. This method is based on the reduction, at low pH, of a colorless ferric complex (Fe^{3+}-tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donating antioxidants (Xu and Chang 2007). The reduction is monitored by measuring the change of absorbance at 593 nm. The working ferric reducing antioxidant power (FRAP) reagent was prepared daily by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ in 40 nm hydrochloric acid and with 1 volume of 20 mM ferric chloride. A standard curve was prepared using various concentrations of $FeSO_4 \cdot 7H_2O$. All solutions were used on the day of preparation. A total of 100 μL of sample solutions and 300 μL of deionized water were added to 3 mL of freshly prepared FRAP reagent. The reaction mixture was incubated for 30 min at 37 °C in a water bath. Then, the absorbance of the samples was measured at 593 nm. A sample blank reading using acetate buffer was also taken. The difference between sample and blank absorbances was calculated and used to calculate the FARP value. In this study, the reducing capacity of the sample tested was calculated with reference to the reaction signal given by a Fe^{2+} solution. FRAP values were expressed as mmol Fe^{2+}/g of sample. All measurements were done in triplicate.

Statistical analysis

Data in triplicate were analyzed by one-way analysis of variance using SPSS 11.5 software package for Windows (SPSS Inc., USA).

RESULTS AND DISCUSSION

Particle sizes of superfine DF powder

The particle size of the superfine DF powder is distributed in a range from 0.38 to 29.91 μm with a mean particle size of 7.06 μm, which belongs to the submicron scale (Figure 1). However, the mean particle size of DF powder before superfine is 73.29 μm. The results reveal that pulverization by mini-type airflow pulverization system can effectively reduce the sizes of the DF particles to a submicron scale; and it is thus feasible to utilize this treatment to manufacture superfine DF powder. Zhu et al. (2010) investigated the particle sizes of ultrafine wheat bran DF powder using multidimensional swing high-energy nanoball-milling. The particle size of the ultrafine DF powder had an average particle size of 343.5 nm. It is then speculated that the reverse phenomena were depending on different materials, treatments, and experimental instruments.

Chemical analysis

As shown in Table 1, the TDF content of WGP was more than 85%, compared with those reported by Valiente et al. (1995) and Bravo and Saura-Calixto (1998) (about 50–60% of TDF), which reveals that the DF prepared by enzymatic method has a fairly

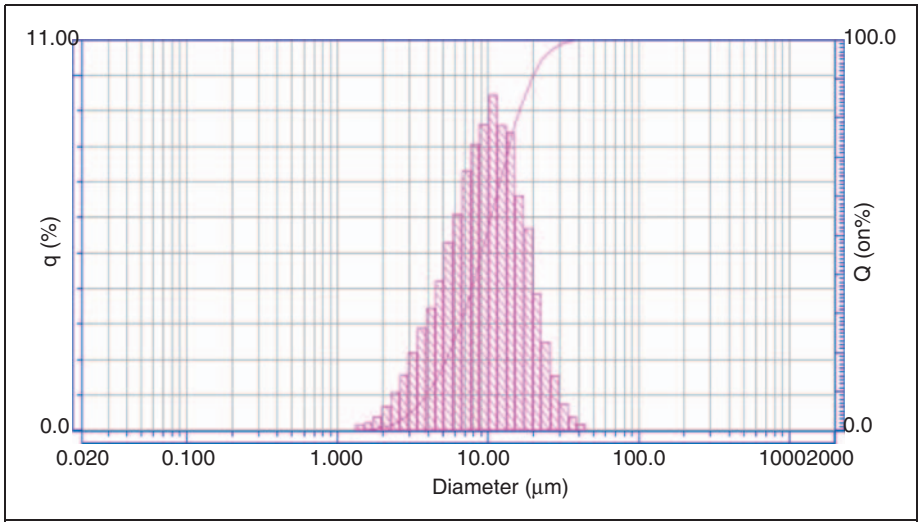

Figure 1. The particle size distribution of superfine DF powder (the abscissa is the diameter (μm) of the particle size; the ordinate *q*% (quantity %) is the particle size distribution, and *Q*% is under size percentage).
DF: dietary fiber.

Table 1. Effects of superfine grinding on the content of DF

Sample	TDF (%)	SDF (%)	IDF (%)	IDF:SDF
Before superfine grinding	86.17 ± 0.10 a	17.27 ± 0.80 b	68.00 ± 0.36 a	3.99 ± 0.20 a
After superfine grinding	82.47 ± 0.46 b	26.50 ± 1.25 a	55.97 ± 0.86 b	2.13 ± 0.13 b

TDF: total dietary fiber; SDF: soluble dietary fiber; IDF: insoluble dietary fiber.
The values represent means of triplicates ± standard deviation.
Values in the same column with different letters are significantly different ($p < 0.05$).

high purity. These researchers obtain 50–60% of TDF using conventional methods. SDF was increased from 17.3% to 26.5% while IDF was decreased from 68.0% to 56.0% after grinding, suggesting that superfine grinding causes a redistribution of fiber components in TDF. The decrease of TDF content after superfine grinding is caused by the degradation of hemicellulose, cellulose and lignin, which are turned into some small molecular compounds (Zhu et al., 2010). A balanced DF composition is suggested to have SDF content higher than 10% (Richard and Leitz Domald, 1989); and consequently, it is an interesting challenge to convert IDF into SDF. It is satisfactory to note that the SDF content of DF powder in this study was higher than 10%, which provides another useful modification method to produce high quality DF.

Physicochemical properties of DF

The WHC, WRC, swelling capacity, OBC, and nitrite ion absorption capacity were studied before and after superfine grinding. All the IDF samples showed increased functional properties after the grinding treatment. As shown in Table 2, the WHC and WRC after superfine grinding were two times higher than before superfine grinding. The swelling capacity, OBC, and nitrite ion absorption capacity after superfine grinding were 1.5 times than before superfine grinding. According to Raghavendra et al. (2004, 2006), the hydration properties of coconut DFs were increased when its particle size was decreased from 1127 to 599 μm. Moreover, Chau et al. (2007) and Zhao et al. (2009) found that the smaller particle size of carrot insoluble DF and ginger powder was associated with higher hydration properties even when their particle sizes decreased to 7.23–8.32 μm because micronization would expose more surface area, polar groups, and other water-binding sites to the surrounding water. However, Zhu et al. (2010) reported the opposite results. They found that the hydration properties of wheat bran DF were decreased after the grinding treatment. It is then speculated that the reverse phenomena were depending on different materials, treatments, and experimental parameters such as stirring, and all

Table 2. Effects of superfine grinding on WHC, WRC, swelling capacity, OBC, and nitrite ion absorption capacity of DF

Sample	WHC (g/g)	WRC (g/g)	Swelling capacity (mL/g)	OBC (g/g)	Nitrite ion absorption capacity (μg/g)
Before superfine grinding	1.23 ± 0.040 b	1.04 ± 0.026 b	5.45 ± 0.066 b	0.97 ± 0.009 b	4.54 ± 0.085 b
After superfine grinding	2.20 ± 0.014 a	1.94 ± 0.076 a	7.67 ± 0.180 a	1.43 ± 0.051 a	5.96 ± 0.150 a

DF: dietary fiber; WHC: water-holding capacity; WRC: water retention capacity; OBC: oil binding capacity.
The values represent means of triplicates ± standard deviation.
Values in the same column with different letters are significantly different ($p < 0.05$).

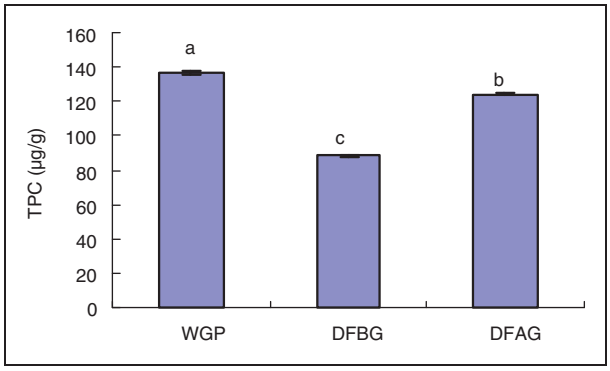

Figure 2. Effects of superfine grinding on TPC.
TPC: total phenolic content; WGP: wine grape pomace; DFBG: dietary fiber before grinding; DFAG: dietary fiber after grinding.

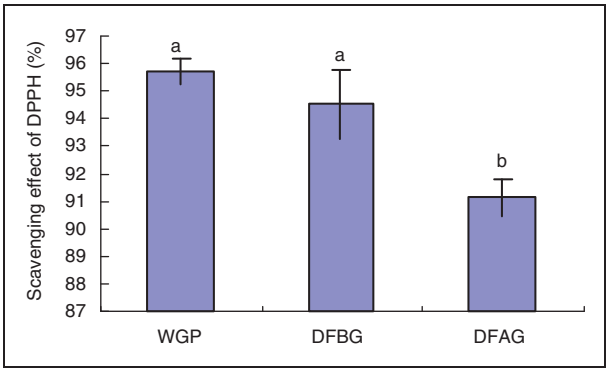

Figure 3. Effects of superfine grinding on radical DPPH scavenging activity.
DPPH: 1,1-diphenyl-2-picrylhydrazyl; WGP: wine grape pomace; DFBG: dietary fiber before grinding; DFAG: dietary fiber after grinding.

of these could alter the physical structure of fibers and thus leads to large changes in its hydration properties (Sangnark and Noomhorm, 2003).

Antioxidant activities of DF

The antioxidant activity was investigated using various established in vitro indexes including DPPH radical scavenging activity, ABTS radial scavenging activity, FRAP assay, and TPC.

The TPC in WGP DF before grinding (DFBG) and after grinding was measured. As shown in Figure 2, the TPC ranked the samples in descending order, as follows: WGP, DF after grinding (DFAG), and DFBG. The phenolic compounds may contribute to overall antioxidant activities. Similar results about wheat bran DF were obtained by Zhu et al. (2010). They revealed that the TPC ranked the samples in descending order, as follows: wheat bran, DFAG, and DFBG.

DPPH radical scavenging activities are shown in Figure 3. The WGP extract had the strongest scavenging activity (96.0%), followed by the DFBG extract (95.7%). DFAG had the lowest activity (91.9%) and the activity of different samples was significantly different ($p < 0.05$). All extracts showed superior antioxidant activity. The DPPH radical scavenging activity was decreased after grinding, a tendency which is an agreement with previous studies suggesting that the DPPH radical scavenging activity is not related with TPC (Yu et al., 2002a, 2002b). There were some other reports presenting the similar phenomena apart from this study. Zhu et al. (2010) suggested that the wheat bran extract had the strong DPPH radical scavenging activity (72.8%), followed by the DFBG extracts (34.5%). DFAG had the lowest activity (24.8%).

The ABTS radical scavenging activity and FRAP of the three samples (Figures 4 and 5) were significantly different ($p < 0.05$) from one to another. WGP showed the strongest activities while DFBG had the weakest among all the samples examined. Both the ABTS radical scavenging activity and FRAP of WGP, DFAG, and DFBG extracts ranked the samples in the same order as the pattern of their TPCs. Thus, a correlation was located among ABTS radical scavenging activity, FRAP, and TPC. The increase of TPC, ABTS radical scavenging activity, and FRAP after grinding is probably on account to the superfine grinding treatment which changed or damaged the fiber matrix, thus

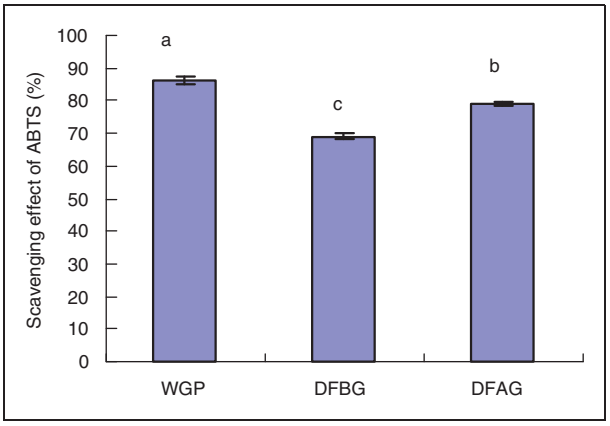

Figure 4. Effects of superfine grinding on radical ABTS scavenging activity.
ABTS: 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonic acid); WGP: wine grape pomace; DFBG: dietary fiber before grinding; DFAG: dietary fiber after grinding.

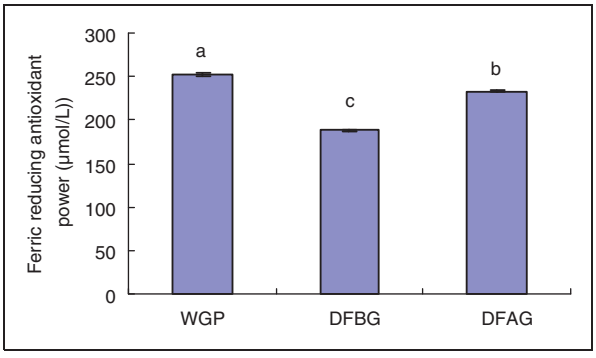

Figure 5. Effects of superfine grinding on FRAP.
FRAP: ferric reducing antioxidant power; WGP: wine grape pomace; DFBG: dietary fiber before grinding; DFAG: dietary fiber after grinding.

causing some phenolic compounds linked or embedded in the matrix to be related or exposed.

In conclusion, the antioxidant activities were related to the TPC except the DPPH radical scavenging activity. During superfine grinding treatment, the ABTS radical scavenging activity and FRAP remarkably increased compared to DFBG, and the DPPH radical scavenging activity decreased compared to DFBG.

CONCLUSIONS

This study has demonstrated that mini-type airflow pulverization system could effectively reduce the particle size of WGP insoluble DF to submicron scale, and the resulting superfine DF has increased functional properties (WHC, WRC, swelling capacity, OBC, and nitrite ion absorption capacity) and redistributed fiber components from insoluble to soluble fractions. Moreover, superfine grinding also causes an increase in ABTS radical scavenging activity, FRAP, and TPC accompanied by a reduction in DPPH radical scavenging activity in the WGP DF.

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