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Effect of superfine grinding on the physicochemical properties and antioxidant activity of red grape pomace powders

Xiaoyan Zhao *, Haitao Zhu, Guixiang Zhang, Weidong Tang

Department of Food Science and Nutrition, School of Hotel Management, University of Jinan, Jinan 250022, China

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ABSTRACT

The objectives of this study were to determine the physicochemical properties and antioxidant activity of red grape pomace powders (GPPs) from winemaking residue with particle sizes of >300, 250–125, 125–70, 70–38, and <18.83 μm. Superfine grinding treatment could decrease bulk density (from 0.40 to 0.21 kg/m^3), tapped density (from 0.51 to 0.31 kg/m^3), and fluidity (repose angle from 33.87 to 56.34° and slid angle from 36.76 to 71.97°, respectively) of GPPs, but improve the solubility (from 9.38 to 48.86%). The extract of GPP with a particle size of <18.83 μm showed that highest total polyphenolic content (757.36 mg GAE/100 g) and flavanol content (19.46 mg CE/100 g) accompanied with the best antioxidant activity through all antioxidant assays ($p < 0.05$). SEM images revealed the shape and surface morphology of GPPs with different sizes. FTIR analysis showed that the superfine grinding did not damage the main structure of phenols as the powder particle size decreased. The results indicated that after superfine grinding treatment, GPPs could serve as a potential resource of natural ingredients for food and nutraceutical application.

1. Introduction

Vitis vinifera L. is an important crop grown in China and worldwide. The total production in the world has exceeded 70 million tonnes [1]. 80% of the grapes are used in winemaking, and 20% of by-products were obtained, including grape stalks (2.5–7.5%), grape pomace (~15% dry; wet up to 25–45%) and grape seeds (3–6%) and yeast lees (3.5–8.5%, yeast lees are the precipitation at the bottom of a wine vat, chiefly including the residual yeast and other particles.) [1]. By-products are rich in nutrient substances, such as sugars, polyphenolics/pigments (red grape pomace), tartarate, fiber, oil and micronutrients [2–4]. In general, the grape seeds consist of basically 40% (w/w) fiber, 16% essential oil, 11% protein, 7% complex phenolic compounds, such as tannins, sugars, minerals, and other substances [5]. The natural pigments, i.e. anthocyanidins and anthocyanins, come from grape skin [6]. However, large amounts of these by-products are often referred to as significant agricultural and industrial wastes and discarded [7,8]. By-products obtained after wine production are a cheap source of antioxidant compounds, which can provide preventative action against diabetes, tumor, cancer, cardiovascular, and neurodegenerative diseases [4,9]. It indicates that the wine making by-products have great potential to be used as functional ingredient in food industry.

Recently, some studies have reported about the investigation of comprehensive utilization of wine making by-products [2,10]. But the researchers chiefly focus on the recovery of some functional components, such as the extraction of total polyphenols, tocopherols, anthocyanins, and flavan-3-ols from grape pomace [2,10,11]. Only a few reports studied the investigation of application of grape marc powder in foods [3]. Sant'Anna et al. [3] found that the grape marc powder was added in fettuccini pasta, which the nutritional properties could be improved, and the contents of total phenolic and tannin in the raw and cooked pasta were similar in the cooking procedure. The reason was that the organelles of vegetable cells could protect most glycosides of phenolics and these compounds were highly bound to the plant matrix [3,11]. Therefore, it is interesting to study the physico-chemical and functional properties of grape pomace powder as a functional ingredient. Generally, fine particles have more application advantages in food industry [12–14].

Superfine grinding technology is applied in functional food processing as a novel technology. The superfine powder of food material has outstanding characteristics, such as surface effect, mini-size effect, quantum effect, macroquantum channel effect, optical property, magnetic property, mechanical property, chemical, and catalytic properties compared to conventional particle materials. Superfine powders have been shown to have oil holding capacity, water holding capacity, and solubility, which is suitable to manufacture instant and convenient foods [13,14]. Recently, Zhang et al. [15] also found that superfine grinding treatment could enhance the antioxidant activities of *Lycium barbarum* polysaccharides. The reason was that the superfine grinding could alter the molecular weight and solution behavior of polysaccharides. It is suggested that superfine grinding parameters should be controlled [16]. However, the effects of superfine grinding treatment

* Corresponding author at: Department of Food and Nutrition, School of Hotel Management, University of Jinan, No. 13 Shungeng Road, Jinan 250002, China.
E-mail address: zhaoxy_2201@163.com (X. Zhao).

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on the physicochemical and antioxidant properties of red grape pomace were unknown yet.

In this study, the objective was to explore the influence of superfine grinding on physicochemical properties and antioxidant activities of red grape pomace powder. The red grape pomace powders with different sizes were prepared, then the main quality chemical composition was analyzed and antioxidant activities in vitro were also determined.

2. Materials and methods

2.1. Materials

Grape pomace was from Urumqi, Xinjiang province, China. Gallic acid, catechin, 1,1′-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS+) and Folin–Ciocalteu reagent were bought from Sigma Chemical Co. (St. Louis, MO). All other chemical reagents were purchased locally and were of analytical grade.

2.2. Sample preparation and particle size measurement

The wet red grape pomace after wine production were dried in a force convection drying equipment at 50 °C, and continued till the water content reached less than 10%. The water content was determined by using AACC method No. 44–19 [17]. The dried grape pomace was milled coarsely by a disc-mill, and then the powders were screened through sieves with >300 and 300–125 μm bore diameter to separate granulates, two different particle size GPPs were obtained. The superfine powders with the particle sizes of 125–70, 70–38 and <18.83 μm were prepared using HMB-701 type micronizer (Zhongkehaoyu Company, Beijing, China) vertically equipped with three rubbing rings on the turntable at around 30 °C. The sample was mainly ground with pressure, collision and abrasion between rubbing rings and inner bottom of pot. The revolution speed of turntable was unfixed at 2500–2800 rpm/min.

2.3. Basic physical property analysis

The particle size was measured by a laser diffraction instrument (Malvern Instruments 2000, UK). Diffraction pattern analysis was carried out in air on a stream of dry powder. Poured bulk density, tapped density, repose and slip angle were measured by using a Powder Integrative Characteristic Tester (BT-1001, Dandong, China) and the water content was determined by gravimetric method [17]. All determinations were performed in triplicate.

2.4. Test procedure for solubility of grape pomace powders

To test the solubility, one gram of different sized samples were accurately weighed, and put into the previously weighed tubes, mixed with exactly 20 mL of distilled water and shaken well. The tubes containing water and samples were weighed again. The tubes were then placed in a reciprocating water bath shaking instrument, and shaken at 30, 40, 50, 60, 70, 80, 90, and 100 °C for different time intervals of 10, 20, 30, 40, 50 and 60 min. Then the tubes were taken out, cooled and centrifuged at 4000 rpm for 20 min. After centrifugation, the supernatant liquids of grape residue powders with different size were transferred to dishes, which were placed in an oven at 70 °C for 60 min, then dried at 100 °C to constant weight. The solubility (S) was calculated from the equation presented:

$$S\% = \frac{A}{W} \times 100\% \quad (1)$$

where W was the weight of the powder (g); A was the weight of supernatant after drying (g).

2.5. Analysis of main chemical components

One gram of GPPs with different size was extracted with 50 ml of 80% methanol in the absence of light at 4 °C for 1 h under stirring. The mixture was filtered through a Whatman No. 1 filter paper. All samples were determined in triplicate. Total polyphenols of grape pomace extracts were determined by Folin–Ciocalteau reagent [18]. 0.5 mL of sample extract was transferred in to a 50 mL volumetric flask and diluted with 4 ml distilled water. Then, approximate 0.5 mL of Folin–Ciocalteau reagent and 0.5 mL of 20% Na_2CO_3 solution were added, agitated, and inculcated at 37 °C for 30 min. The absorbance of sample was determined on the UV-2401PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 760 nm against a blank prepared with distilled water. Gallic acid was used as standard for calibration. The results were expressed as gallic acid equivalents in mg/100 g dry matter (dw).

The total flavanol content of the grape pomace extracts was estimated using a modified vanillin method [19]. 1 mL of grape pomace extracts and 5 mL of vanillin solution (4% in 1 N HCl in MeOH) were mixed. Then the mixture was hold at 30 °C for 20 min. The absorbance was measured at 500 nm using a UV-2401PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The results were interpreted according to the calibration curve expressed as milligrams of catechin equivalent per 100 g^{-1} dry matter.

2.6. Evaluation of the antioxidant capacity

The extracts of grape pomace powders with different size were prepared, as described in Section 2.5. Absorbance measurements were read on a UV-2401PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.6.1. DPPH radical scavenging assay

Antioxidant activity of grape pomace powder extracts was assessed by scavenging DPPH radical, which was carried out according to the method of Zhang et al. [15] with some modifications. DPPH was dissolved in ethanol at concentration of 0.2 mM. 2 mL of freshly prepared DPPH and 2 ml of extracts with different size particles were thoroughly mixed. The mixture was shaken well, allowed to stand for 30 min in the dark, and the reading was taken at 517 nm. The blank consisted of 2 ml ethanol solution of DPPH and 2 ml ethanol. The capability to scavenge DPPH radical was calculated as follows:

$$\text{DPPH scavenging effect } (\%) = \left[A_0 - \frac{(A - A_b)}{A_0}\right] \times 100 \quad (2)$$

where A_0 is the absorbance of DPPH solution without sample; A is the absorbance of the test sample mixed with DPPH solution and A_b is the absorbance of the sample without DPPH solution. IC50 was expressed as the concentration of 50% of DPPH radical-scavenging activity.

2.6.2. ABTS radical scavenging assay

Radical cation scavenging capacity of grape pomace powder extracts was examined against ABTS, which was described according to the method of Zhang et al. [15] with some modifications. The reaction between 5 mL of 7 mM ABTS solution and 2.45 mM potassium persulfate produced ABTS radical cation. Then, the mixture was stood in the dark at room temperature for 14 h. Before usage, this stock solution was diluted with phosphate buffer solution (PBS, pH 7.4). In the assay, 100 μL of grape pomace powder extracts and 400 mL of ABTS working solution were vortexed for 10 s in a reaction tube. Thereafter, the reaction mixture was allowed to stand at 30 °C for 6 min. The absorbance was recorded at 734 nm. The ABTS scavenging effect was calculated as follows:

$$\text{ABTS scavenging effect } (\%) = \left[A_0 - \frac{(A - A_b)}{A_0}\right] \times 100 \quad (3)$$