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The Influence of Particle Size Reduction on Functionality, Polyphenol Extractability and In Vitro Digestibility of Grape Pomace

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Abstract

Grape pomace (GP) is rich in dietary polyphenols and dietary fiber (DF) and has great potential to serve as a food ingredient to improve the nutritional value of food products. Particle size reduction of GP is necessary for polyphenol extraction and analysis, DF determination and food product fortification. This study evaluated the impacts of particle size reduction on water holding capacity (WHC) and oil binding capacity (OBC), polyphenol extractability and *in vitro* digestibility of GP from four different grape varieties. Dried GPs were ground and separated into four fractions using a set of standard sieves. The particle size of each fractions was determined by a laser particle size analyzer. Total polyphenols (TP), total anthocyanin (TA), total flavonoid (TF) and condensed tannin (CT) of GP fractions with different particle sizes were determined. Total dietary fiber (TDF) without adjusting non-digestible protein was determined as an indicator of digestibility. Results show that both WHC and OBC decreased with particle size reduction; Reducing particle size of GP significantly increased extractability of TP, TF, TA and CT, particularly, when GP particle size was reduced from 489 μm to 209 μm with exception of TA. Particle size reduction also resulted in decreased TDF which indicates increased digestibility of GP. The results suggest that particle size of GP has tremendous impacts on composition analysis and applications of GP. Therefore, GP must be ground fine enough for accurate analysis of its chemical composition. To compare the polyphenol and DF composition data from different labs, samples have to have similar particle size. To maximize the beneficial effects of GP in food, the particle size of the pomace should be well controlled.

Keywords: *Grape Pomace; Particle Size; Functional Properties; Polyphenol Extractability; Dietary Fiber*

Abbreviations

GP: Grape Pomace; DF: Dietary Fiber; WHC: Water Holding Capacity; OBC: Oil Binding Capacity; TP: Total Polyphenol; TA: Total Anthocyanin; TF: Total Flavonoid; CT: Condensed Tannin; TDF: Total Dietary Fiber; IDF: Insoluble Dietary Fiber; SDF: Soluble Dietary Fiber

Introduction

Grape is one of the oldest fruit crops domesticated by humans. The uses of grape in making wine, beverages, jelly, and other products, has made it one of the most economically important fruits worldwide. Grape pomace (GP) is a by-product/waste of grapes from wine production. It consists mainly of peels (skins), seeds and stems and is starch free [1]. GP accounts for approximately 25% of the grapes crushed. The GP is rich in different types of polyphenols. Lu and Foo (1999) isolated 17 kinds of polyphenol from grape pomace [2]. The health benefits of GP polyphenols have been the great interest of researchers, food and nutraceutical industry because of their potential antioxidant property and preventive effects against many diseases such as heart diseases and increased blood cholesterol of GP polyphenols [3-5]. In addition to their nutritional importance, polyphenols also play important roles in the functional qualities of foods, such as flavor and appearance. The polyphenol compositions of GP in different areas of the world were reported by many researchers, and most of the results were incomparable because of the polyphenol composition of GP varies with grape variety, growing condition and wine making practice [6]. In addition, sample preparation methods such as solvents and extraction temperature for polyphenols extraction and analysis will also affect the results [7,8]. However, the effect of particle size reduction on polyphenol extraction and analysis is rarely reported.

GP is also rich in non-digestible components including pectin, cellulose, Klason lignin [9-11] which are the major components of dietary fiber (DF). The total dietary fiber (TDF) contents of GP was reported to be up to 75% of the dry mass [9,12]. DF and plant polyphenol consumption has been shown inverse association with cardiovascular diseases, obesity, diabetes, gastrointestinal disorder and some types of cancers [13]. Although the healthy reputation of dietary fiber continues to grow, national data consistently show that both children and adults consume less than one-half of the recommended daily intakes of dietary fiber [14], and more than 90% of Americans do not meet the fiber recommendation [15]. Therefore, it is important to explore new source of dietary fiber and use them to increase the dietary fiber contents of some commonly consumed foods. GP has potential to serve as a good source of dietary fiber for food fortification [16].

Inclusion of GP in food products could result in functional foods with beneficial effects of dietary fiber and grape polyphenols. To include GP in the food product, size reduction is necessary to reduce the coarse and grainy texture. Mechanical treatment, such as grinding/milling, is usually used to reduce particle size of cereal fibers for better acceptance of the final products, but it may affect the bioaccessibility of polyphenols in fiber rich materials such as grape pomace because of the complexation of polyphenols with fiber [17]. The functional and physicochemical properties of polyphenol and DF rich materials can also be influenced by particle size reduction [18,19]. The objective of this study was to investigate the influence of the particle size reduction of GP on the water holding and oil binding capacity, polyphenol extractability and the *in vitro* digestibility of GP powders from four cultivars of grapes.

Materials and Methods

Grape pomace preparation

Pomaces from Cabernet Franc, Cabernet Sauvignon, Muscadine Noble (Mus Noble), and Muscadine Carlos (Mus Carlos), Cabernet Franc (Cab Franc) and Cabernet Sauvignon (Cab Sauvignon) were obtained from two wineries in North Carolina, United States. They were dried for 24 hours at 78 - 80°C in an Isotemp 285A vacuum oven (Fisher Scientific, USA), then ground into powder using a coffee grinder. A set of standard sieves (Fisher Scientific, USA) were used to separate the powder into fractions of different particle size ranges (20 - 40 mesh, 40 - 60 mesh, 60 - 80 mesh, and 80 - 100 mesh). The fractions of different particle size were stored in moisture proof plastic bags at 4°C for further analysis.

Particle size analysis

The average sizes of different fractions of ground pomace were determined by laser diffraction (LD) technology using a Laser Particle Size Analyzer (Microtrac, Montgomeryville, PA, USA). In which, a laser beam is passed through a well-dispersed particle sample and particle size is measured by detecting the intensity of the scattered light produced. The data is collected continuously throughout the measurement, is analyzed and put through an algorithm using Microtrac's innovative modified Mie scattering theory and produces accurate particle size distributions for both spherical and non-spherical particles. The equipment also gives the particle size distribution of each fraction.

Water holding capacity and oil binding capacity of grape pomace

The water-holding capacity (WHC) and oil-binding capacity (OBC) of GP fractions of different particle sizes are the two important properties which affect the quality of food products. These properties were determined by the method described by Raghavendra and colleagues [18]. Briefly, 1.00g of GSF was weighed in a pre-weighed 15 ml test tube. 10 ml of DI water or 10 ml of commercial vegetable oil (100% Pure vegetable Oil, all Natural) was added to the tubes. Samples were kept at room temperature for 18 hours, then centrifuged at 3000 g for 20 minutes. After centrifugation, the supernatants were completely decanted and the residues were weighed. Measurements were triplicated and the results were expressed as the mean weight of water or oil absorbed by one gram dried sample.

Polyphenol extraction and analysis

Polyphenol in each fractions was extracted as described in our previous study using 70% ethanol [20].

Determination of total extractible polyphenol (TP)

The TP concentration of the extract was determined by Folin-Ciocalteu method [21] with small modification and expressed as mg gallic acid equivalent (GAE)/g GP. Briefly, 20 µl of properly diluted extract was mixed with 1.28 ml of DI water and 100 µl of Folin-Ciocalteu reagent in a test tube. The mixture was kept at room temperature for 8 minutes, then the 0.6 ml of 10% sodium carbonate was added and mixed. The mixture was kept in dark at room temperature for 2 hours. The absorbance was measured at 765 nm using a Genesys™ 10 Spectrophotometer (Spectronic Unican, NY, USA). The TPC concentration was calculated using the calibration equation developed using gallic acid standard solutions:

$$Y = 1.1775X + 0.0233 \quad R^2 = 0.9944 \qquad (1)$$

Where X is gallic acid concentration. TPC in grape pomace was expressed as gallic acid equivalents (mg GAE/g sample).

Determination of total anthocyanins

The TA was determined by AOAC method 2005.02 [22] and calculated as cyanidin-3-glucoside equivalents for the samples. Briefly, the extracts were mixed thoroughly with 0.025 M potassium chloride (pH 1.0) in a known dilution. The absorbance of the mixture was measured at 515 and 700 nm using distilled water to zero the spectrophotometer. The extracts were then combined with 0.4 M sodium acetate buffer (pH 4.5), and the absorbance was measured at the same wavelengths. The absorbance of the diluted sample (A) was calculated by equation (2):

$$A = (A515 - A700)\ \text{pH } 1.0 - (A515 - A700)\ \text{pH } 4.5 \qquad (2)$$

The anthocyanin content was calculated as the total of monomeric anthocyanin pigment from equation (3), shown below:

$$\text{Anthocyanin (mg/g)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1) \qquad (3)$$

Where A is the absorbance of the diluted sample and DF is the dilution factor. MW and ε in this formula correspond to the predominant anthocyanin in the sample. Since the sample composition was unknown, the pigment content was calculated as cyanidin-3-glucoside, where MW = 449.2 and ε = 26,900.

Determination of extractable total flavonoids (TF)

The TF was determined by the aluminum chloride ($AlCl_3$) colorimetric method described by Yang and colleagues [23] and expressed as mg catechin/g sample. Briefly, an aliquot of 0.25 ml of GP extract was mixed with 1.25 ml of distilled water and 75 µl of 5% sodium nitrite. After 6 minutes, 150 µl of 10% aluminum chloride were added and standing for 5 minutes prior mixed with 0.5 ml of 1M sodium hydroxide and 775 µl of distilled water. The absorbance of the solution was determined at 510 nm. The calibration curve was established using (+)-catechin solutions (50 - 400 µg/ml). The TF concentration in the extract was calculated by equation (4):

$$Y = 311.03X - 7.3965; \quad R^2 = 0.9955 \qquad (4)$$

Where, Y is absorbance, X is catechin concentration. The result of total flavonoid contents in the GP extract were expressed as milligram catechin equivalent (mg CE)/ml extract.

Determination of total condensed tannin (TCT)

The condensed tannin content was measured colorimetrically using Vanillin-HCl Assay by the method of Hagerman [24]. The total condensed tannin was calculated using the standard curve developed using a set of catechin standard solutions with concentrations 0.05, 0.12, 0.18, and 0.24 mg/ml, respectively (Equation 5). The results were expressed as mg catechin/g sample.

$$Y = 0.670X + 0.006; \quad R^2 = 0.998 \qquad (5)$$

Where, Y is absorbance, X is catechin concentration. The condensed tannin concentrations of the GP extracts were expressed as milligram catechin equivalent (mg CE)/ml extract.

In vitro digestibility of grape pomace

The *in vitro* digestibility of grape pomace samples with different particle sizes was evaluated by total dietary fiber (TDF) without adjustment of non-digestible protein GP. The TDF was determined by AOAC method 985.29 [25] using a TDF-100A kit (Sigma-Aldrich, St. Louis, MO), according to manufacturer's instruction.

Data analysis

The data for WHC, OBC and polyphenol extractability were analyzed by post-ANOVA Duncan multiple range comparison. The relationship of between TDF and GP particle size was analyzed by linearly regression analysis.

Results and Discussions

Particle size distribution of different GP fractions

The distribution of GP particles in the fraction of 20 - 40 mesh showed two Bell shaped curves, one from 16 - 357 μm, another started 419 μm and ended 2000 μm and 78% of particles fell in the range of 88 - 2000 μm with average of 689 μm (Figure 1A). The particle distributions of other fractions were all in one bell shape curves (Figure 1B-1D). For 40 - 60 mesh fraction, 87.5% of the GP particles fell in the range of 170 - 999.5 μm with average particle size of 486 μm. For 60 - 80 mesh fraction, 92.6% were in the range of 74 - 419 μm, with average particle size of 209 μm. For 80-100 mesh fraction, 94.02% of particles were in the range of 18.5 - 248.9 μm with average particle size of 104 μm. These data indicate that each fraction contains particles of different sizes, but most of particles had size close to the average of the fraction.

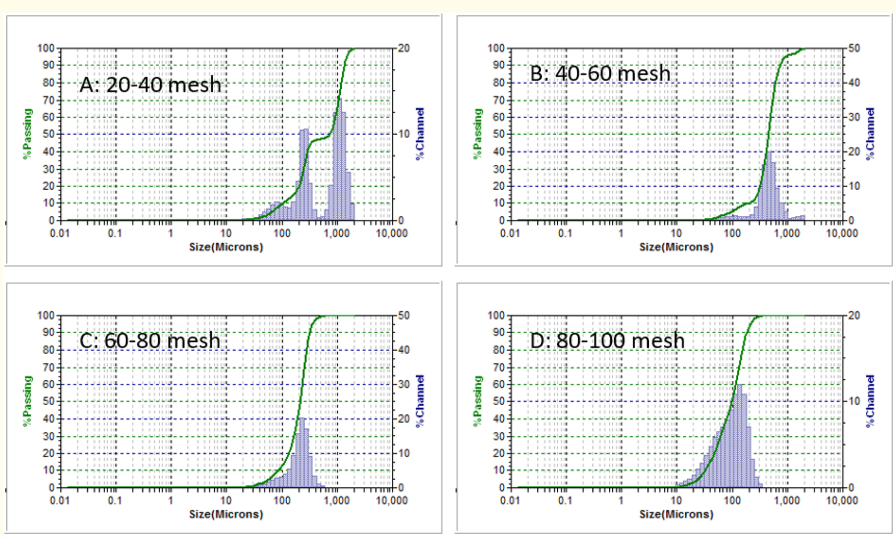

Figure 1: *Particle size distribution of different fractions of grape pomace. A-The fraction of 20 - 40 mesh; B-The fraction of 40 - 60 mesh; C-The fraction of 60 - 80 mesh; D-The fraction of 80 - 100 mesh.*

Effect of GP particle size on water holding capacity and oil binding capacity

Water holding capacity (WHC) and oil binding capacity (OBC) are two important functionalities of a food ingredient. Overall, both WHC and OBC of GP negatively correlated to particle size reduction. That is, reduction of particle size resulted in decrease of WHC and OBC

(Figure 2). However, when the average particle size decreased from 689 to 486 um, the WHC and OBC of Muscadine Carlos, Muscadine Noble and Cabernet Sauvignon increased, but the WHC and OBC of Cabernet Franc decreased. Further particle size reduction resulted in linearly decrease in both WHC and OBC of Cabernet GPs and Muscadine Noble GP, but WHC and OBC of Muscadine Carlos GP were less affected. Similar result was observed for sugarcane bagasse [26] and wheat bran [27]. In addition, more cell materials such as fat and protein should be released out as particle size decreasing. High fat content makes the fraction more hydrophobic thus reducing WHC. The high fat ingredient also absorbs less oil when they are mixed with oil.

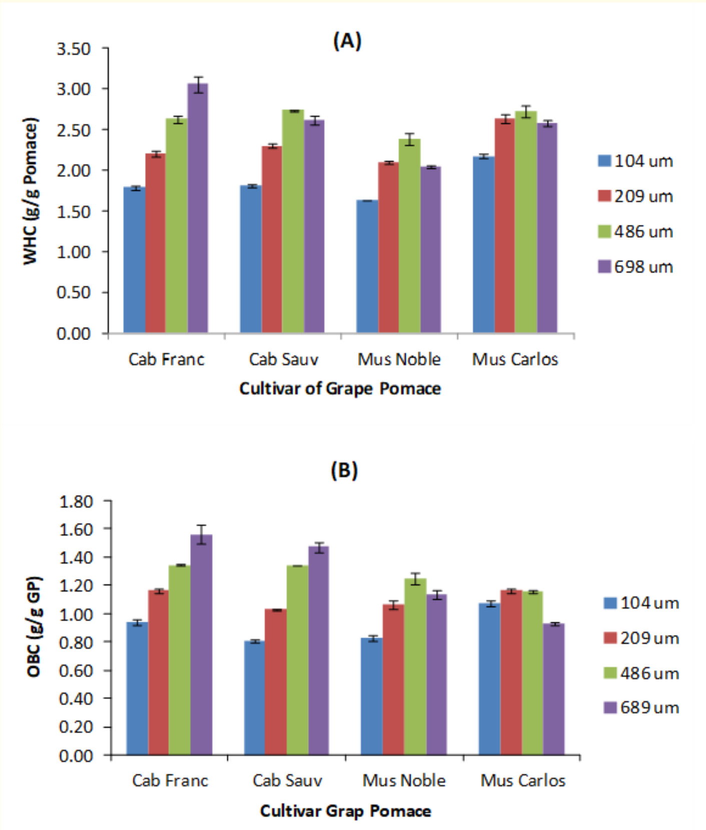

Figure 2: *Effects of particle size of grape pomace on its (A) water holding capacity and (B) oil binding capacities.*

Effect of particle size on polyphenol extractability of GP

Polyphenol extractability of different fractions of ground pomace was evaluated by determination of total extractible polyphenol (TP), total anthocyanin (TA), total flavonoid (TF) and condensed tannin (CT) contents of each GP fraction. At same particle size, the pomace of Cabernet Franc had lowest TP and TA, the pomace of Cabernet Sauvignon showed highest TP, TF and CT, while the pomaces of Muscadine Noble showed highest TA but lowest CT contents (Figure 3). No TA was detected in any fraction of Muscadine Carlos pomace because Muscadine Carlos is a light color grape variety (Figure 3C). The higher TF and CT contents of Cabernet Franc and Sauvignon pomaces might be attributed to fact that there were more seeds wrapped by the skins because the CT content in grape skin was reported to be much lower than that in seeds [28], while anthocyanins were reported to be the main phenolic compounds in the red/dark color grape skins [29-31].

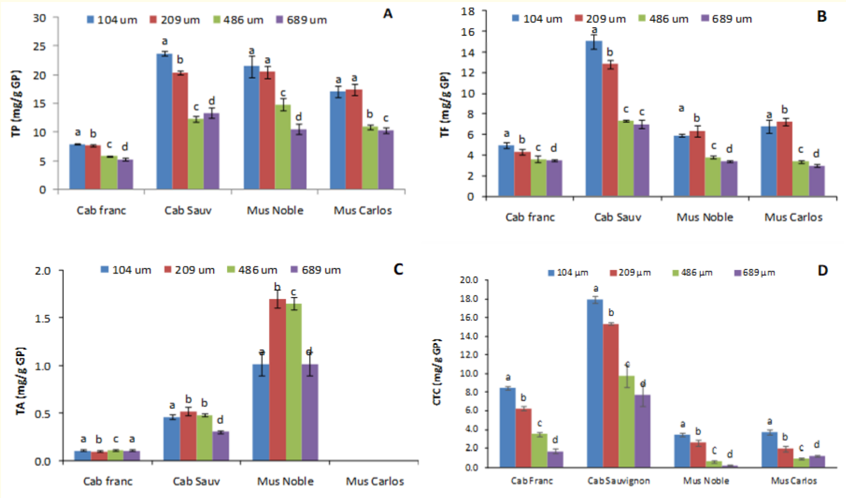

Figure 3: *Polyphenol extractability and composition of fraction of GP with different particle sizes (A- Total polyphenol, B- Total Flavonoids, C- Total Anthocyanin and D- Total Condensed Tannin) (Data with different superscripts for the same GP are significantly different at P < 0.05).*

Reducing particle size of grape pomace significantly increased extractability of total polyphenol (Figure 3A), total flavonoid (Figure 3B) and total condensed tannin (Figure 3C). From particle size 689 um to 486 µm, the change of extractible TP and TF were small but statistically significant (P < 0.05) with exception of Muscadine Noble GP, but the extractible TP, TF and CT increased sharply when GP particle size was reduced from 489 µm to 209 µm (Figure 3A, 3B and 3D). The extractible TA increased remarkably when the average particle size was reduced from 689 µm to 486 µm. There was a small but significant TA increase from 489 µm to 209 µm, but further particle size reduction resulted in decreased extractable TA (Figure 3C). The highest extractability of TA was achieved at particle size 209 um for all GP samples except Cabernet Franc pomace. The majority portion of GP polyphenols has been reported to be highly polymerized condensed tannin, and some polyphenols form complex with fiber, and are non-extractable unless strong acidic treatments are applied [17]. The results of this study show that the extractability of highly polymerized tannin can be greatly improved by mechanically reducing particle size of GP (Figure 3D). The study of Makanjuola on the polyphenol extractability of tea and ginger also found that more TP and TF were extracted when particle size decreased from 1.18 mm to 0.71 mm, but further reduction of particle size to 0.425mm, the extractability of both TP and TF decreased for tea, but increase for ginger [32]. Our results are in good agreement with what reported by Brewer and colleagues where fine fraction of wheat bran had higher in phenolic acid, flavonoid, anthocyanin, and carotenoid contents than coarse fraction [33].

Effect of particle size on total dietary fiber of GP

TDF without adjustment of non-digestible protein measures the total non-digestible matters of GP. Therefore, the digestibility of GP samples of different particle sizes was evaluated by TDF containing non-digestible protein. Reducing particle size decreased the TDF contents of all grape pomace, but the decrease of TDF of GP with particle size varied with grape varieties (Figure 4). The TDF of Muscadine Carlos was least affected by the particle size as shown by the smaller coefficient constant (Figure 4A), followed by that of Cabernet Sauvignon pomace and Cabernet Franc pomace (Figure 4C and 4D), while the TDF of Muscadine Noble was most affected by particle size (Figure 4B). The observed results may be caused by the influence particle size on the measurement TDF. It was reported that hemicellulose values for wheat samples ground through a 60-mesh screen of Wiley Mill were 20% lower than samples ground through a 20-mesh screen [34]. Some very small particles may pass through the crucible filter used for TDF measurement. Therefore, it is important to specify the particle size of the material when AOAC method 991.43 is used to determine the dietary fiber profile of plant materials and the pore size of crucibles used for fiber determination. The decrease of TDF due to particle size reduction indicates the increased overall digestibility. For same grape cultivar, reducing particle size resulted in increasing digestibility of GP by different digestive proteases (Figure 4). At same particle size, the pomace of Muscadine Carlos exhibited lowest digestibility, while the pomace of Muscadine Nobel showed highest digestibility except when the average particle size was 689 µm. One study reported that particle size of oat hull and sugar beet did not affect broiler performance, but coarse grinding increases gizzard development and reduced nutrient digestibility in young birds [35]. Another study found that the feed conversion ratio was worse on the coarsely ground high crude fiber diet than on the finely ground diet in growing-finishing pigs [36]. Therefore, for better digestibility, the fiber rich food and feed ingredients need to be finely ground, but for weight control/loss, the ingredient should be coarsely ground.

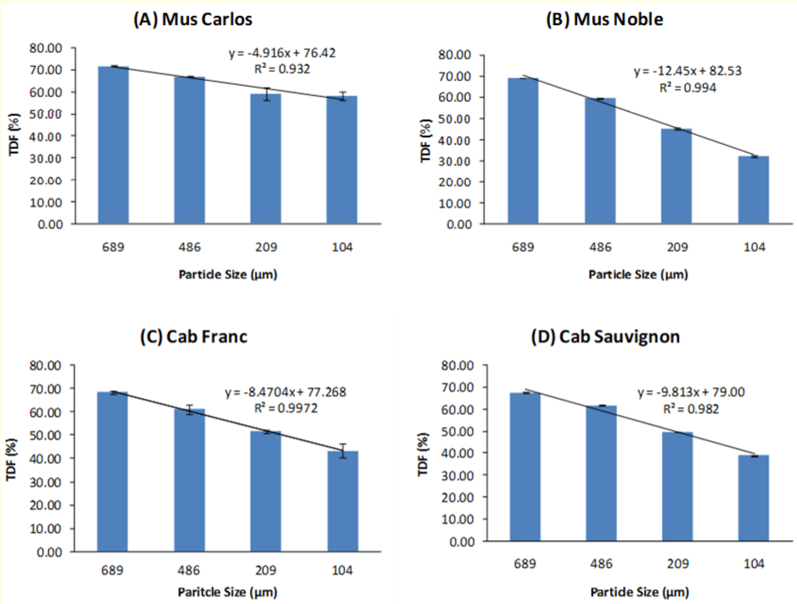

Figure 4: *Total dietary fiber contents of grape pomace at different particle sizes.*

Conclusions

This study indicates that particle size reduction has significant impact on the functional properties, polyphenol extractability and digestibility of grape pomace. Reducing particle size of GP mechanically can greatly improve the extractability of polyphenols, which will be very important for GP polyphenol production. However, particle size reduction may also result in undesirable taste to food fortified with GP powder because increased concentration and accessibility of flavonoids and condensed tannins which usually are responsible for the bitter and astringent taste of many food products [37]. To accurately quantify chemical composition such as polyphenol composition, the GP has to be sufficiently ground to achieve the necessary fineness. To achieve comparable results of extractable polyphenols and TDF, the particle sizes of different GP samples have to be in the same range. According to the results of this study, the fraction with average particle size 209 µm or smaller is recommended for polyphenol extraction and quantification. To increase the polyphenols accessibility and GP digestibility, GP also should be finely ground. In the situation where lower digestibility is desired, GP should not be ground too fine.

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Conflict of Interest

There are no financial interests or conflicts of interest.

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