PREPARATION OF GRAPE POMACE POWDERS AND ANALYSIS OF THEIR NUTRITIVE COMPOSITIONS

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Abstract
Grape pomace (GP) is produced in large amounts worldwide, leading to waste of resources and environmental pollution. Using grapes grown in eastern China, the main nutrients and polyphenols in grape seeds (GS), skin (GSK), and GP powders were determined by conventional chemical composition analysis and HPLC-MS/MS. The antioxidative activity of a GP polyphenol extract was identified using DPPH and hydroxyl radical scavenging assays and reducing power assay. GSK and GP contained less total dietary fiber than GS. The total polyphenolic content of GS was significantly higher than that of GSK and GP. The hydrogen- and electron-donating activities of the GP polyphenol extract were superior to those of vitamin C.

Keywords:
nutritional content; polyphenolic content; grape seeds; grape skin; grape pomace; antioxidative activity

Introduction

Grapes are popular cultivated fruits in the world, with approximately 80% being processed for wine production each year [1]. One byproduct of the winemaking industry is grape pomace (GP), which is made up mainly of grape skin (GSK) and grape seeds (GSs) and accounts for approximately 20%–30% of the total weight of fresh grapes [2]. In 2016, 26.7 billion liters of wine were produced worldwide, generating approximately 6.5 million to 11.5 million tons of grape residues [3]. However, only a small amount of GP is recycled as fertilizer and feed, and the rest is thrown away as garbage, causing a great waste of resources and environmental pollution [4,5]. Grape dregs are in fact rich in nutrients, such as polyphenols, tartaric acid, malic acid, proteins, vitamins, GS oil, and dietary fiber [6]. The polyphenols are the most valuable components of GP, and there has been great interest in their extraction owing to their health benefits [7]. A previous study showed that compared with butylated hydroxytoluene, a polyphenol extract had much higher antioxidative activity, with 50% inhibiting concentration (IC_{50}) values of 20.5, 52.5, 566.4, and 16.8 mg/L for the 2,2-dipheny-1-picrylhydrazyl (DPPH), superoxide anion, hydroxyl, and 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt free radicals, respectively [8]. Wang et al. [9] evaluated the *in vitro* antitumor activity of resveratrol from GP, demonstrating its inhibitory effect against HeLa, A549, and PC-3 cells, with IC_{50} values of 128.29, 108.35, and 25.31 μmol/L, respectively. In another study, GS extracts inhibited the proliferation of MDA-MB468 human breast cancer cells by 90%–100%. In GP, polyphenols such as phenolic acids, tannins, flavones, and coumarin provide the anticancer effect and have other potential bioactivities [10,11] . Nie et al. [12] extracted polyphenols from GP and found that they had inhibitory effects on the food-borne pathogenic bacteria *Staphylococcus aureus*, *Shigella*, *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli,* with the effect on *S. aureus* and *Salmonella* being the greatest.

In recent years, consumers have been paying increasing attention to the nutritional value and health effects of foods. GP has gained interest as a raw material to produce flour products owing to its high nutrient content and bioactivities, and products produced with grape powders would have a richer nutritive value and comprehensive physiological functions. The grape variety, maturity, and growth conditions (e.g., soil, climate, rainfall, and planting techniques) are important factors affecting the composition and content of grape dregs [13]. In view of this, this study was carried out to systematically evaluate the nutritive composition of GP powders using the standard curve method and chromatography. Additionally, the antioxidative activity of a GP polyphenol extract was investigated. The results from this study should provide a reference for the further development and comprehensive utilization of GP.

Materials and methods

Materials and chemicals

Fresh grapes (Cabernet Sauvignon, 2019 crop) were obtained from Huailai city (Hebei, China). DPPH and Folin–Denis reagent (made up of 20 g of sodium tungstate, 4 g of phosphomolybdic acid, 10 mL of phosphoric acid (all purchased from Nanjing Chemical Reagent Co., Ltd., Jiangsu, China), and 150 mL of water, condensed and refluxed for 2 h, cooled, and diluted to 200 mL), potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid, ferric chloride ($FeCl_3$), hydrogen peroxide (H_2O_2), petroleum ether, acetone, aluminum nitrate, ferrous sulfate ($FeSO_4$), high-temperature-resistant alpha-amylase solution (30 U/mg), and glycosylase solution (100 U/mg) were purchased from Shanghai Ruji Bio-Technology Co., Ltd. (Shanghai, China). Alkaline protease solution (100 U/mg) was purchased from Shanghai Lanji Bio-technology Co., Ltd. (Shanghai, China). Tris-(hydroxymethyl) aminomethane (Tris), gallic acid, and rutin were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Folin–Ciocalteu (FC) phenol reagent and tannic acid were purchased from Aladdin (Shanghai, China). All other chemicals used were of analytical grade.

Preparation of the various grape powders

The Cabernet Sauvignon grapes were first fermented with 1 wt% distillery yeast at 25 °C for 7 days. Then, using a laboratory-scale extruder (Shanghai Yulushiye Instrument Co., Ltd., Shanghai, China), the GP was collected and washed four to five times with water. By contrast, the GSK and GS were gathered manually. The GP, GSK, and GS samples were then placed outdoors for dehydration to a water content of approximately 65 wt%, following which they were transferred to an air-dry oven at 50 °C for 80 h. The dried GP, GSK, and GS samples were then individually crushed using a laboratory-scale pulverizer (Shanghai Zhikai Powder Machinery Manufacturing Co., Ltd., Shanghai, China) and passed through an 80 mesh screen to gather powders with a particle diameter of >40.52 µm (Fig. 1).

Fig. 1. (a) Grape seeds powder; (b) grape skin powder; (c) grape pomace powder; (a') defatted wine grape seeds powder; (b') defatted wine grape skin powder; (c') defatted grape pomace powder. *Source: Author's.*

Chemical compositions of the various grape powders

The moisture, ash, protein, and lipid contents of the GP, GS, and GSK powders were assayed using the AACCI-approved methods 44–15, 08–01, 46–11, and 30–10, respectively [14]. The total sugar content was determined using a direct titration method [15]. The total dietary fiber (TDF), insoluble dietary fiber, and soluble dietary fiber contents were measured using the enzymatic-gravimetric method [16]. The minerals and trace elements in the powders were assayed using inductively coupled plasma atomic emission spectrometry (Optima 2100DV, PE Co., USA), with the following conditions: power, 1300 W; injection rate, 1.5 mL/min; nebulizer flow rate, 0.8 L/min; auxiliary gas flow rate, 0.2 L/min; and combustion gas flow rate, 15 L/min.

Polyphenolic compositions of the various grape powders

The tannin content of the three groups of grape powders was determined by sodium tungstate–

phosphomolybdic acid-based colorimetric analysis at a wavelength of 765 nm, whereas the total polyphenolic and proanthocyanidin contents were measured using the FC phenol reagent and molysite catalysis-based colorimetry, respectively [17,18]. The total flavonoid content was determined using the aluminum nitrate–sodium nitrite method [19]. The polyphenolic compositions of the various samples were analyzed by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) [20]. To obtain the polyphenol extracts, the GS, GSK, and GP samples were individually extracted in methanol in a 25 °C shaking water bath (SHA-C, Shanghai, China) for 24 h in a dark environment. Then, after centrifugation of the extract at 6000 rpm for 15 min, the supernatant was concentrated *in vacuo* at 30 °C and made up to 25 mL with Crypt-grade methanol. The chromatographic conditions were as follows: mobile phase A, 0.1% acetic acid in water (volume fraction); mobile phase B, acetonitrile; flow rate, 0.4 mL/min; column temperature, 30 °C; and injection volume, 5 µL. The gradient elution conditions were as follows: 0–15 min, 10% B; 15–20 min, 10%–35% B; 20–23 min, 35%–90% B; 23–24 min, 90% B; and 24–30 min, 90%–10% B. The MS conditions were as follows: electrospray ion source; ion source temperature, 325 °C; dry gas flow rate, 10 mL/min; sheath gas flow rate, 11 L/min; sheath gas temperature, 350 °C; and capillary voltage, 3000 V. The multi-reaction detection method was used.

Main mineral elements assay

Microwave digestion: Various amounts of the GSK (0.5015, 0.5019, 0.5023, 0.5029, and 0.5031 g), GS (0.5009, 0.5015, 0.5017, 0.5021, and 0.5028, g), and GP powders (0.5011, 0.5018, 0.5019, 0.5024, and 0.5029 g) were individually weighed and added to a digestion tank together with 4 mL of concentrated nitric acid. The mixtures were shaken under the following microwave digestion program: initial heating to 100 °C for 10 min, a hold for 1 min, heating to 180 °C for 8 min, and maintaining at this temperature for 25 min. After digestion, the sample solution was washed a few times with ultrapure water in a 25 mL plugged colorimetric tube and then diluted to the mark. A reagent blank was also tested.

Preparation of a polyphenol extract from grape pomace for antioxidative activity assay

To extract the polyphenols, 10 g of GP powder was first degreased with petroleum ether for 12 h. Then, the sample was mixed with 70% ethanol in a 1:5 ratio (sample:ethanol, w/v) and ultrasonic-assisted extraction was carried out six times (40 min each time) using an ultrasonic power of 250 W and extraction temperature of 30 °C. All extracts were then collected and filtered, all extracts were then collected and filtered, and an equal volume of each filtrate (50 mL) was vacuum concentrated.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the GP polyphenol extract was determined using a previously published method [21]. In brief, 2 mL of different concentrations of the sample in ethanol was respectively mixed with 2 mL of 2 mmol/L DPPH in ethanol and incubated at ambient temperature for 30 min. The absorbance of the DPPH-reacted sample (A_i) was then measured at 517 nm using a UNICO spectrometer (Shanghai, China). That of the control (A_c) containing ethanol instead of the sample was also measured under the same experimental conditions. The absorbance of the original unreacted sample in ethanol was recorded as A_j. Finally, the DPPH radical scavenging activity of the sample was calculated using the following formula: DPPH radical scavenging activity (%) = $[1 – (A_i – A_j)/A_c] \times 100\%$. Vitamin C at the same concentrations as the sample was also tested for comparison of the antioxidative activities.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of the GP polyphenol extract was measured using a previously reported method [22]. In brief, 2 mL of sample solutions at different concentrations was mixed with 1 mL of 9 mmol/L $FeSO_4$ and 1 mL of 9 mmol/L salicylic acid–ethanol, and the reaction was then initiated by the addition of 1 mL of 8.8 mol/L H_2O_2 solution. Following 30 min of incubation in a 37 °C water bath, the absorbance of the sample (A_x) was measured at 510 nm using the UNICO spectrometer. Controls of 2 mL of deionized water in place of the $FeSO_4$ and salicylic acid–ethanol solution (A_{x0}) and of 2 mL of deionized water in place of the sample solution (A_0) were also measured under the same experimental conditions. A system without H_2O_2 added was used as the reference solution when measuring A_0, whereas deionized water was used as the reference for determining A_x and A_{x0}. The hydroxyl radical scavenging activity of the sample was calculated as follows: hydroxyl radical scavenging activity (%) = $[A_0 – (A_X – A_{X0})/A_0] \times 100\%$. Vitamin C at the same concentrations as the sample was also tested for comparison of the antioxidative activities.

Reducing power assay

The reducing power of the GP polyphenol extract was determined according to a previously published method [23]. In brief, 2.5 mL of samples at different concentrations was mixed with 2.5 mL of PBS (pH 6.6) and 2.5 mL of a 10 mg/mL $K_3Fe(CN)_6$ solution and the mixture was incubated for 20 min in a 50 °C water bath. Then, after the solution had cooled, 2.5 mL of 100 mg/mL trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. Thereafter, 2.5 mL of water and 0.5 mL of 0.1% $FeCl_3$ were added to the supernatant and the absorbance at 700 nm was measured using the UNICO spectrometer. Vitamin C at the same concentrations as the sample was also tested for comparison of the antioxidative activities.

Statistical analysis

All experiments were conducted in triplicate, and the results are expressed as the mean ± standard deviation. Dunnett's T3 test was applied for multiple comparisons and differences were statistically significant at $p < 0.05$. SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical evaluations, and OriginPro 8.6.0 was used for the construction of the graphs.

Results and discussion

Chemical compositions of the various grape powders

Table 1 shows the moisture, ash, protein, and crude fat contents of the various grape powders. The main component of the three types of powder was dietary fiber, the amounts of which ranged from 59% to 68%, with GS power having a significantly richer content than GSK powder ($p < 0.01$). The main components of dietary fiber are non-starch polysaccharides and plant cell walls, which have bioactivities of maintaining normal intestinal function and lowering blood pressure. Notably, dietary fiber contains neutral polysaccharides, uronic acids, Klason lignin, and resistant proteins. Most of the TDF in GP powder was insoluble, whereas soluble dietary fiber (mainly neutral polysaccharides and uronic acids) accounted for only 13% of the TDF. Neutral polysaccharides and Klason lignin accounted for 80% of the TDF, and resistant proteins accounted for only 5.4%.

Table 1. Chemical compositions of various grape powders

Component	GS powder (% DW)	GSK powder (% DW)	GP powder (% DW)	Significance
Crude protein	8.75 ± 0.03^{C}	16.31 ± 0.02^{A}	13.62 ± 0.01^{B}	**
Crude fat	20.92 ± 0.06^{A}	10.66 ± 0.10^{C}	17.46 ± 0.04^{B}	**
Ash	2.98 ± 0.11^{C}	3.95 ± 0.05^{B}	6.02 ± 0.14^{A}	**
Moisture	3.69 ± 0.21^{C}	4.51 ± 0.18^{B}	5.86 ± 0.25^{A}	**
TDF	68.53 ± 0.29^{A}	59.38 ± 0.33^{C}	65.20 ± 0.28^{B}	**
Total carbohydrate	57.04 ± 0.48^{A}	50.25 ± 0.55^{Bc}	51.66 ± 0.71^{Bb}	*

All data are expressed as the mean ± standard deviation (n = 3). * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively. Different Latin letters within the same column indicate significant differences among the grape powder samples (lowercase and uppercase letters represent $p < 0.05$ and $p < 0.01$, respectively). GS, grape skin; GSK, grape skin; GP, grape pomace; DW, dry weight; TDF, total dietary fiber.

The results also indicated that the dried powders still contained a certain amount of moisture. The water content has a direct relationship with the water-absorbing capacity of GP powder and wheat flour after mixing, which in turn affects the formation and stability of the gluten protein network structure in the dough. The highest ash content was GP powder, followed by GSK powder, and the lowest ash content was contained in GS powder, which is consistent with previous results [24]. Ash contains different minerals and trace elements, and as a cofactor for various enzymes, it also participates in the physiological processes that form bones, hemoglobin, and cytochromes, and maintains osmotic pressure and acid–base balance in the body. Proteins, which are major food constituents, are an essential nutrient for the human body where they have many physiological functions. The crude protein amounts in the GS, GSK, and GP powders were 8.75%, 16.31%, and 13.62%, respectively, which was also consistent with the results of previous studies [25]. In general, the crude protein content of GP, GS, and GSK was higher than that of ordinary cereal seeds (e.g., that of corn seeds powder is 8.60%). GS powder had the highest crude fat amount (20.92%). This was consistent with reports that the energy of GS is 22.64 MJ/kg because of its high fat content [26]. By contrast, GSK powder had higher protein and lower fat contents than GS powder. The amounts of the main mineral elements in the various powders are shown in Table 2. GP powder was rich in K, Na, Ca, and Mg, which are the major physiologically beneficial elements, and contained Fe, Cu, Zn, Mn, and Cr, which are the trace elements that are necessary for the human body. Aside from their health and nutritional

value, K, Ca, Mg, Fe, and Mn play important roles in human growth and development, hematopoietic function, and immune function. The anticancer element Se was also detected in the GS powder. By contrast, the amount of harmful Pb was low in all three powders, and that of Cd was not detected at all. The Pb content in all the samples did not exceed the Chinese National standards for "Green food – dried fruits" (NY/T 1041-2010) and "Green food – temperate fruits" (NY/T 844-2010), which stipulate a Pb amount of ≤1 mg/kg and a Cd amount of ≤0.05 mg/kg.

Table 2. Amounts of minerals and trace elements in various grape powders

Elements	GS powder (mg/g)	GSK powder (mg/g)	GP powder (mg/g)	Significance
K	7.361 ± 0.21^{C}	22.746 ± 0.33^{A}	18.678 ± 0.11^{B}	**
Na	0.273 ± 0.01^{Bc}	0.364 ± 0.00^{Aa}	0.318 ± 0.02^{Bb}	*
Ca	5.575 ± 0.24^{A}	2.277 ± 0.03^{C}	3.785 ± 0.32^{B}	**
Mg	1.286 ± 0.41^{ABa}	0.778 ± 0.00^{Bb}	1.129 ± 0.02^{Aa}	
Fe	0.025 ± 0.01^{C}	0.079 ± 0.00^{A}	0.061 ± 0.00^{B}	**
Zn	n.d.	n.d.	0.001 ± 0.00	
Cu	0.019 ± 0.00^{C}	0.092 ± 0.00^{A}	0.078 ± 0.00^{B}	**
Mn	0.002 ± 0.00	n.d.	n.d.	
Cr	0.002 ± 0.00^{C}	0.004 ± 0.00^{A}	0.003 ± 0.00^{B}	
Se	0.002 ± 0.00	n.d.	n.d.	
Pb	0.001 ± 0.00	n.d.	n.d.	
Cd	n.d.	n.d.	n.d.	

All data are expressed as the mean ± standard deviation (n = 3). Significance: * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively. Different Latin letters within the same column indicate significant differences among the powder samples (lowercase and uppercase letters represent $p < 0.05$ and $p < 0.01$, respectively). GS, grape skin; GSK, grape skin; GP, grape pomace; n.d., not detected.

The contents of minerals and trace elements were quite different among the three powder samples. The amounts of the major elements K and Na and trace elements Fe, Cu, and Cr were significantly higher in GSK powder than in GS powder ($p < 0.01$), especially K and Na, which were as high as 22.75 and 0.365 mg/g, respectively. GS powder was rich in Ca and Mg, the amounts of which (5.576 and 1.287 mg/g, respectively) were significantly higher than those of GSK powder ($p < 0.01$). Additionally, the amounts of Se, Mn, and Zn in GS powder reached 2, 2, and 1 mg/kg, respectively. These results are quite different from the Se content of grapes reported in the literature [27–29], which may be related to the grape species studied and their different origins. Depending on the contents of minerals and trace elements in GSKs and GSs, different processing methods can be considered. Because GS is rich in trace elements, such as Fe, Cu, and Cr, it can be considered to produce health-related products, but attention should be paid to whether it is contaminated.

Polyphenolic compositions of the various grape powders

The HPLC-MS/MS chromatograms of grape polyphenols in the various samples are shown in Fig. 2, and the results of the quantitative analysis are presented in Table 3. In this study, GSK powder and GS powder were selected as representatives to analyze. GSK and GS contained a large amount of polyphenols (Table 3). GSK contained the highest amounts of flavonol (quercetin, morin, myricetin, etc.) and benzoic acid polyphenols (vanillic acid, syringic acid, gallic acid, etc.), whereas it had only small amounts of flavan-3-ol (catechin, epicatechin, epicatechin gallate, epigallocatechin gallate) and cinnamic acid polyphenols (i.e., only 0.5 mg/kg coumaric acid). In the GS sample, approximately 80% of the non-anthocyanin polyphenols were flavan-3-ol polyphenols, among which those extracted by methanol reached 707.48 mg/kg and were significantly greater in amount than the benzoic acid and flavonol polyphenols. The GS powder also contained low concentrations of cinnamic acid. These data are consistent with the results of other studies [20,30,31]. It should be noted that in the GS sample, the quercetin content was much lower and the isoquercitrin amount was much higher than that presented in previous reports[20,32]. This was likely because the polyphenolic composition in GP depends on many factors, such as the grape variety, growth climate, geographic environment, fermentation time, and maturity [33,34] Among the various polyphenols in GSK, the flavonols and benzoic acids were in the highest amounts, followed by theflavan-3-ol, cinnamic acid, and stilbene compounds. By contrast, the GS sample had the highest amount of non-anthocyanin polyphenols, especially flavan-3-ol polyphenols. In the GS sample, the contents of benzoic acid and flavonol polyphenols were lower than that of flavan-3-ol polyphenols and similar to those of the GSK sample. The GS sample contained only trace amounts of cinnamic acid and stilbene polyphenols. In the GP

sample, the flavonol with the highest content was myricetin, followed by morin, quercetin, isorhamnetin, and kaempferol, and its content of flavonols was higher than that of the GS sample. In the GSK and GS samples, catechins and epicatechins were predominant, accounting for 96%–100% of the total flavan-3-ol polyphenols. Moreover, the contents of catechins and epicatechins in the GS sample were significantly higher than those in the GSK sample ($p < 0.05$), which is consistent with other studies [20].

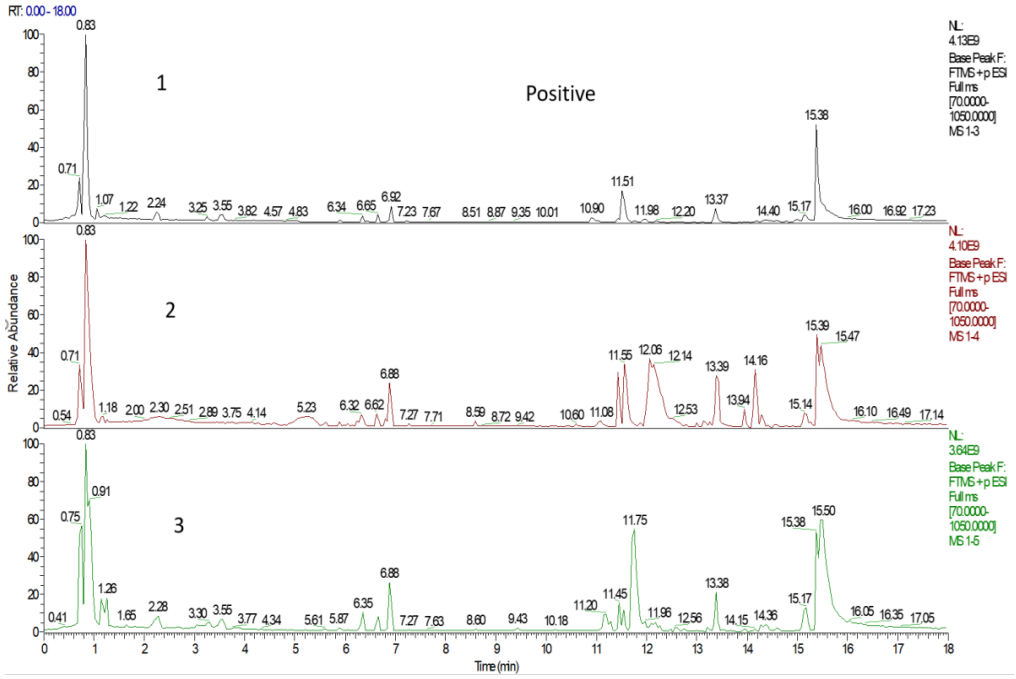

Fig. 2. HPLC-MS/MS chromatograms of polyphenols in various grape powder samples. 1: Grape seeds; 2: grape skin; 3: grape pomace. *Source: Author's*

Table 3. Polyphenolic compositions of various grape samples.

Component	GS (mg/kg)	GSK (mg/kg)	Significance
Epicatechin	405.51 ± 5.04^{A}	87.70 ± 1.12^{B}	**
Epicatechin gallate	7.85 ± 0.28^{A}	2.99 ± 0.34^{B}	**
Epigallocatechin gallate	2.00 ± 0.23^{a}	1.84 ± 0.37^{a}	
Gallic acid	152.57 ± 2.21^{A}	65.53 ± 0.78^{B}	**
Coumaric acid	1.24 ± 0.04^{A}	0.50 ± 0.04^{B}	**
Vanillic acid	93.65 ± 1.15^{B}	125.00 ± 2.21^{A}	**
Syringic acid	3.56 ± 0.23^{B}	40.89 ± 0.76^{A}	**
Catechin	316.80 ± 3.07^{A}	69.62 ± 0.45^{B}	**
Myricetin	59.98 ± 1.14^{B}	250.40 ± 2.54^{A}	**
Morin	10.92 ± 0.28^{B}	33.64 ± 1.25^{A}	**
Quercetin	1.64 ± 0.16^{B}	33.17 ± 0.89^{A}	**
Isorhamnetin	0.89 ± 0.01^{B}	11.24 ± 0.67^{A}	**
6-Gingerol	0.15 ± 0.02^{A}	0.04 ± 0.002^{B}	**
Kaempferol	1.13 ± 0.11^{B}	3.21 ± 0.13^{A}	**
Luteolin	0.28 ± 0.02^{B}	0.73 ± 0.01^{A}	**
Isoquercitrin	10.74 ± 0.30^{A}	0.02 ± 0.005^{B}	**

All data are expressed as the mean ± standard deviation (n = 3). Significance: * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively. Different Latin letters within the same column indicate significant differences among the grape samples (lowercase and uppercase letters represent $p < 0.05$ and $p < 0.01$, respectively). GS, grape skin; GSK, grape skin.

Studies have shown that GSK and GS contain a certain amount of resveratrol, with that in red GSK being 1.11 – 12.3 mg/100 g, which was not consistent with the results of this study. This is closely related to the solvent and pretreatment method used for extracting the GP. In addition to resveratrol, GP also contains a certain amount of the stilbene compound piceid, whereas the contents of other stilbene compounds are very low [20].

These phenolic substances give wine its color and various flavor characteristics and constitute the important factors of wine quality [35,36]. The contents of total phenols, total flavonoids, tannins, and proanthocyanidins in the GS powder were higher than those in the GSK and GP powders (Table 4). The amounts of total phenols in the GS, GSK, and GP powders were the highest, at 92.68, 56.43, and 87.21 mg/g, respectively, followed by the proanthocyanidins and tannins. The proanthocyanidins give wine its bitter and astringent characteristics and are an important taste substance and key quality component in wines [37]. They can also combine with anthocyanins to form stable condensates during the wine aging process, thereby ensuring the stability of the wine color [38]. Additionally, proanthocyanidins are strong antioxidants, having the ability to absorb oxygen free radicals, and their antioxidative activity has been found to be 20 times that of vitamin C and 50 times that of vitamin E [39]. With regard to tannins, Zhang et al.[40] studied their effects on gluten protein structure, dough property, and bread quality and found that they not only could break down the disulfide bonds but also had positive effects on the dough properties and bread quality. Subsequently, those authors used this property to investigate new, safe, and efficient additives of flour [41].

Table 4. The amounts of the main phenolics in various grape samples

Items	Standard equation	Standard plasmids	GS	GSK	GP
Total polyphenols (mg GAE/g DW)	Y = 0.0133x – 0.0045 R^2 = 0.9995	Gallic acid	92.68 ± 0.56	56.43 ± 0.35	87.21 ± 0.69
Flavonoids (mg RE/g DW)	Y = 0.0827x + 0.0006 R^2 = 0.9994	Rutin	9.86 ± 0.12	2.50 ± 0.25	7.20 ± 0.32
Tannins (mg TAE/g DW)	Y = 0.057x + 0.0256 R^2 = 0.9911	Tannic acid	19.28m ± 0.31	13.49 ± 0.55	17.91 ± 0.18
Proanthocyanidins (mg CE/g DW)	Y = 0.0048x + 0.0226 R^2 = 0.9956	Proanthocyanidin	84.50 ± 0.46	37.21 ± 0.57	71.08 ± 0.38

All data are expressed as the mean ± standard deviation (n = 3) in units of mg/g DW. DW, dry weight; GS, grape skin; GSK, grape skin; GP, grape pomace; GAE, gallic acid equivalent; RE, rutin equivalent; TAE, tannic acid equivalent; CE, cyanidin equivalent.

Antioxidative activity of the polyphenol extract from wine grape pomace

Currently, three authoritative methods are used for assaying antioxidants in herbal ingredients; namely, the DPPH and hydroxyl radical scavenging assays and the reducing power assay [42]. As shown in Fig. 3, the GP polyphenol extract had a strong capability in scavenging DPPH free radicals, exhibiting higher antioxidative activity than vitamin C, even at a low mass concentration, with the activity increasing as its mass concentration increased. At the mass concentration of 2 μg/mL, the DPPH free radical scavenging activity of the extract was 85.80%, which was 54.3 times higher than that of vitamin C (1.58%). The hydroxyl radical scavenging assay, which tests the H-donating capacity of the sample, revealed the polyphenol extract to be much more active in trapping free hydroxyl radicals than vitamin C. Additionally, according to the Fe ion-reducing power assay, the electron-donating effectiveness of the polyphenol extract was also significantly higher than that of vitamin C.

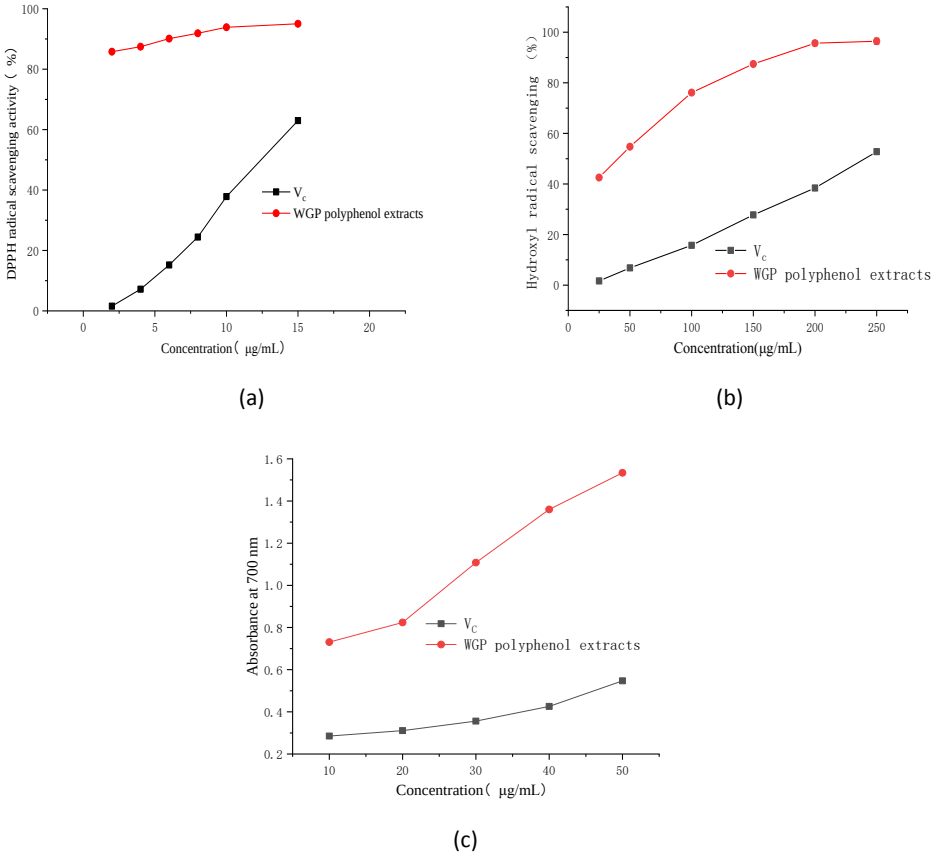

Fig. 3. Antioxidative activities of a wine grape pomace (WGP) polyphenol extract and of vitamin C (V_c). (a) DPPH radical scavenging activity; (b) Hydroxyl radical scavenging activity; (c) Reducing power. *Source: Author's.*

Impact

Grape pomace is identified as a low-value by-product, and primarily used as animal feed component or feedstock for fertilizer in many small- and medium-sized enterprises, which underestimates the promising utilization value of grape pomace. Currently, grape pomace is receiving increased attention for its higher phenolic compounds content, numerous studies have supported grape phenolic compounds are associated with prevention of chronic degenerative diseases (atherosclerosis, cancer, cardiovascular disease and type 2 diabetes, among others), many researchers add grape pomace into food, the reasons of adding GPP to different food are varied, from improving the sensory qualities to increasing the nutritional value.

Grape powders have been proved to have strong antioxidant properties in this study. The polyphenols and minerals contents are rich, which can be added to flour products as nutritional enhancement agents in the food industry and can also be used as antioxidants to extend the shelf life. Therefore, the effect of grape powders on rheological properties and microstructure of wheat dough will be studied in the future, to verify whether grape powders are excellent raw material for food processing, to investigate the appropriate addition level of different food types, to make fully utilize of grape powders to process new products such as bread, biscuit and cake, etc., which can not only improve food nutritional value, but create economic benefits, as well as solve the problem of environmental and resource waste caused by improper disposal of grape residue.

Conclusions

Compared with ordinary cereal seeds and skins, GP is rich in nutrients (protein, fat, carbohydrates, etc.) and mineral and trace elements (K, Ca, etc.), and its total phenolic content is high. We have also verified that the antioxidative activity of its polyphenol extract was far superior to that of vitamin C. According to previous reports, polyphenols play a positive role in the physical and chemical properties of flour and consequently the quality of baked goods. Thus, the results of this study can be effectively applied to the development of food and health products supplemented with GP, effect of grape powders on rheological properties and microstructure of wheat dough will be studied in the future, thereby breathing new life into this wasted winery byproduct and reducing its negative impact on the environment.

Conflict of interest

There are no conflicts to declare.

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