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Phenolic compounds and antioxidant properties of different grape cultivars grown in China

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ABSTRACT

Skins and seeds of 18 grape cultivars belonging to Oriental and North American *Vitis* Species/hybrids, and *Vitis vinifera* were analysed for health beneficial properties. Four phenolic compound parameters (total phenols, flavonoids, flavan-3-ols and anthocyanins) and three antioxidant property parameters (DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS [2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)] radical scavenging and FRAP (ferric reducing antioxidant power)) were measured. Principal component analysis (PCA) was used for this evaluation and results showed that both phenolic compounds and antioxidant properties in the seeds and skins varied among the cultivars investigated. *V. vinifera* "Cabernet Sauvignon" had the highest values of phenolic compounds and antioxidant properties in seeds followed by Muscadines, while the lowest appeared in the Oriental *Vitis* species. As expected, these values of the Euro-Asian or Euro-American hybrids fell between the parents. However, far less variation of these values was observed in the skins among different grape cultivars investigated. Interestingly, even the total phenolic contents in the berries of two cultivars are similar, distributions of phenolic compounds in seeds and skins varied greatly among them. Additionally, significant correlations among different antioxidant assays in both seeds and skins were observed. These antioxidant properties were also found highly correlated to the main phenolic compounds.

1. Introduction

Grape is one of the largest fruit crops growing world wide. According to the FAO statistical database, grape production reached 66 million tons in 2005 (FAOSTAT, 2005). Grape has been appreciated for their rich content of phenolic compounds such as gallic acid, catechin and resveratrol, and a wide variety of procyanidins. A wide range of biological activities of these phenolic compounds has recently been reported: inhibition oxidation of human low-density lipoproteins (Frankel, Waterhouse, & Teissedre, 1995), antioxidant properties and radioprotective effects (Castillo et al., 2000), prevention of cataract (Yamakoshi, Saito, Kataoka, & Tokutake, 2002), antihyperglycemic effects (Pinent et al., 2004), modulation of the expression of antioxidant enzyme systems (Puiggròs et al., 2005), anti-inflammatory effects (Terra et al., 2007) and therapy of cancer (Nandakumar, Singh, & Katiyar, 2008).

Each year the processing of grapes for wine and juice globally leaves behind an estimated amount of at least 10 million tons of press residues (Maier, Andreas, & Dietmar, 2009). Berry skins and seeds are where most phenolic compounds accumulate. For this reason, grape residue extract has become popular in recent years as a nutritional supplement. However, although the literature abounds with reports about phenolic compounds and antiradical activity of grape seeds or skins, there are very few reports comparing distributions of phenolic compounds between seeds and skins among different species and cultivars. Knowledge of the phenolic compound distribution between seed and skin in a berry will contribute to a more comprehensive assessment of the berry biological activities.

It has been well known that the grape nutritional qualities are affected by environmental, cultural, and post-harvesting conditions, but genotype is the determined factor leading to the variation (Connor, Luby, Tong, Finn, & Hancock, 2002; Prior, Cao, Martin, Sofic McEwen, & O'Brien, 1998; Proteggente et al., 2002). Knowledge of health-beneficial nutrition distribution among wild grape species and less used cultivars are very important for improving grape nutritional properties by breeding. European grapes commercially spreads around the world and their phenolic compounds and antiradical activities have been well studied but East Asia grape germplasms have yet to be fully explored for their nutritional activities.

Objectives of this study were therefore to screen several important Chinese grape species for their phenolic compounds

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and antioxidant profiles and make a comparison with the European and muscadine grapes. In the mean time, the other objectives are to improve the assessment of these compounds and to better understand their distributions in grape seeds and skins.

2. Materials and methods

2.1. Materials

2.1.1. Plant materials

A total of 18 cultivars belonging to five Oriental *Vitis* species, *Vitis vinifera*, three Euro-Asian Hybrids, one Euro-American Hybrid, and musadine grape (*Vitis rotundifolia*) grown in different locations of China were used for this study (Table 1). Ripe berries were collected from commercial vineyards. The "Cabernet Sauvignon" pomace was collected three weeks after skin-fermentation at around 23 °C.

The grape seeds and skins were separated manually from berries or pomace, and were immediately freeze-dried in a Freeze Drier (LGJ-18, Ruibang Xinye Corporation, Beijing, China). Dried specimens were stored in vacuum-packaged polyethylene pouches at −20 °C until analysis.

2.1.2. .Sample preparation

Freeze dried grape seeds were crushed and then defatted with petroleum ether at a ratio of 10:1 (v/w). After 3 h of shaking at room temperature, the liquid was separated from the solid by vacuum filtration through a sintered glass filter (Pyrex, porosity 10–15 μm). The defatted process was carried out twice and the solid residue was evenly distributed over a tray and kept in the dark for evaporation of petroleum ether. The final defatted grape seed powders were put into a mortar containing liquid nitrogen and ground into a powder as fine as possible for subsequent analysis.

Grape skins were ground sufficiently with a stainless-steel grinder (FW-135, Taister Corporation, Tianjin, China; 30(0.6 mm)–200(0.075 mm) sieve size) to pass 60 sieve size (0.25 mm). The ground samples were used for subsequent analysis.

2.1.3. Chemicals

Folin and Ciocalteu's phenol reagent (2 N), Gallic acid (⩾98%, UV, HPLC), Procyanidin (⩾95%), Malvidin-3,5-diglucoside (⩾98%), Rutin (⩾98%), 2,4,6-tripyridyl-s-triazine (TPTZ) (⩾99%), 2,2′-azino-bis-(3-ethylben zothiazoline-6-sulfonic acid) diammonium salt (ABTS) (⩾98%), 2,2-diphenyl-1-picrylhy-drazyl (DPPH) (⩾97%) were obtained from Sigma–Aldrich (St. Louis, MO). Sodium carbonate and vanillin were purchased from Merck (Darmstadt, Germany). 6-Hydroxy-2,5,7,8-tetramethylchro-man-2-carboxylic acid (Trolox) (⩾99%) was obtained from Alexis (Axxora, Switzerland). All other chemicals and solvents were of analytical reagent grade and purchased in China.

2.2. Methods

2.2.1. Extraction of phenolic compounds

Phenolic compounds were extracted from grape seeds and skins using methanol/water/acetic acid (70:29:1, v/v/v) which proved to be the most effective solvent for this study (unpublished data). 0.2 g of freeze-dried and prepared seeds or skins were weighed into 50 ml centrifuge tube with 8 ml solvent in an orbital shaker at 300 rpm for 100 min at 25 °C. After pouring out the supernatant, the precipitate was re-extracted with 8 ml of the same solvent two more times. The supernatant were combined in a 50 ml tube, and centrifuged (Beckman Coulter Ltd, Palo Alto, CA, USA) at 5000 rpm for 20 min. Finally the supernatant was collected and stored at −20 °C in dark until further analysis (normally within 2d). Extractions were performed in three replicates for all samples.

2.2.2. Photometric determination of total phenols, total flavonoids, total flavan-3-ols, and total anthocyanins

The total phenolic content in grape seeds or skins was determined by the Singleton and Rossi (1965) method on a UV–Vis double beam UNICO UV-2800 spectrometer (UNICO, New York, USA). Gallic acid (GA) was used as standard and expressed as gallic acid equivalents (mg GAE/g DM, mg gallic acid/g of dry defatted matter) through the calibration curve of gallic acid. The linearity range of the calibration curve was 50–1000 μg/ml ($r = 0.9998$). The total flavonoid content was determined using the colourimetric method described previously by Dewanto, Wu, Adom, and Liu (2002). The results were calculated and expressed as micrograms of rutin equivalents (mg RAE/g DM) using the calibration curve of rutin. The linearity range of the calibration curve was 100–1000 μg/ml ($r = 0.9992$). The total flavan-3-ols content was determined by the Vanillin assay (Sun, Ricardo-da-Silva, & Spranger, 1998) using procyanidin as the standard and expressed as procyanidin equivalents (mg PAE/g DM) through the calibration curve of procyanidin. The linearity range of the calibration curve was 10 to 250 μg/ml ($r = 0.9998$). The total anthocyanin content was determined following the procedure described by Lohachoompol, Srzednicki, and Craske (2004) using Malvidin-3,5-diglucoside as the standard and expressed as Malvidin-3,5-diglucoside equivalents (mg MAE/g DM) through the calibration curve of Malvidin-3,5-diglucoside. The linearity range of the calibration curve was 100–1000 μg/ml ($r = 0.9995$). All analyses were replicated twice with means ± SD being reported.

2.2.3. Measurement of antiradical properties

2.2.3.1. Free radical-scavenging activity on DPPH. The DPPH assay was based on the slightly modified method of Brandwilliams, Cuvelier, and Berset (1995). Briefly, 100 μl of sample was diluted with 400 μl phosphate buffered saline(PBS) at pH 7.4, and then 100 μl of the diluent was added to 3.9 ml methanolic solution of DPPH (0.0025 g/100 ml CH_3OH). After 60 min at RT in the dark, the absorbance at 515 nm was recorded to determine the concentration of the remaining DPPH. The percentage inhibition of DPPH of the test sample and known solutions of Trolox were calculated by the following formula: $\%\text{Inhibition} = 100 \times (A_0 - A)/A_0$, where A_0 was the beginning absorbance at 515 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the test sample at 515 nm. The calibration curve between %Inhibition and known solutions of Trolox was then established. The radical scavenging activities of the test samples were expressed as trolox equivalent antioxidant capacity (μM TE/g DM) on their percentage inhibitions. Trolox standard solutions were prepared at a concentration ranging from 100 to 1000 μM.

2.2.3.2. Free radical-scavenging activity on ABTS. For ABTS [2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)] assay, a procedure modified from Re et al., 1999 was used. Trolox or samples (20 μl) were diluted with 40 μl PBS at pH 7.4, and then the diluent was added to 2.0 ml of diluted $ABTS^+$ solution, and absorbance readings at 734 nm were taken at 30 °C exactly 10 min after initial mixing. The percentage inhibition of $ABTS^+$ of the test sample and known solutions of Trolox were calculated by the following formula: $\%\text{Inhibition} = 100 \times (A_0 - A)/A_0$. where A_0 was the beginning absorbance at 734 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the test sample at 734 nm. The calibration curve between %Inhibition and known solutions of Trolox was then established. The radical-scavenging activity of the test samples were expressed as trolox equivalent antioxidant capacity (μM TE/g DM) on their percentage inhibitions.

Table 1
Phenolic compounds in seeds and skins among 18 grape cultivars belonging to seven species and interspecific hybrids.

Species/cultivars	Year	Total phenols (mg GAE/g DM)[a,b]		Total flavonoids (mg RAE/g DM)[a,b]		Total flavan-3-ols (mg PAE/g DM)[a,b]		Skin anthocyanins(mg MAE/g DM)[a,b]	Color
		Seeds	Skins	Seeds	Skins	Seeds	Skins		
Black Pearl	2007	17.24 ± 0.45 ab	39.24 ± 0.57 k	11.12 ± 0.14 ab	28.24 ± 0.99 k	7.86 ± 0.39 abc	7.81 ± 0.78 k	19.89 ± 0.54 J	Black
Black Pearl	2008	18.34 ± 0.05 ab	40.20 ± 0.97 k	9.81 ± 0.59 ab	23.19 ± 0.65 J	6.58 ± 0.47 ab	6.50 ± 0.62 i	18.68 ± 0.08 J	Black
Sangye	2007	24.99 ± 0.89 d	41.21 ± 1.24 l	19.41 ± 1.18 e	31.84 ± 1.64 l	14.94 ± 0.91 e	13.42 ± 0.71 o	23.05 ± 0.46 k	Black
Sangye	2008	24.64 ± 0.44 d	38.64 ± 0.48 k	17.51 ± 1.05 de	27.94 ± 0.43 k	14.11 ± 0.33 e	12.75 ± 0.44 o	19.91 ± 0.07 J	Black
Purple grape	2007	15.79 ± 0.48 a	27.56 ± 1.01 i	9.96 ± 0.94 ab	19.74 ± 0.77 i	5.75 ± 0.56 a	7.22 ± 0.84 J	7.04 ± 0.03 de	Purple
White grape	2008	24.84 ± 0.69 d	16.88 ± 0.34 c	15.40 ± 0.24 cd	12.64 ± 0.45 cd	13.16 ± 0.81 e	9.60 ± 0.95 l	ND	White
V.quinquangularis Rehd.									
Mao grape	2007	18.84 ± 0.66 b	28.09 ± 0.46 J	15.22 ± 0.91 cd	19.92 ± 0.89 i	8.43 ± 0.64 bc	5.89 ± 0.58 h	9.44 ± 0.77 h	Red
V. amurensis Rupr.									
Zuosanyi	2007	22.11 ± 0.91 c	24.44 ± 0.93 fg	13.78 ± 0.53 bc	15.48 ± 0.47 f	10.86 ± 0.71 d	5.22 ± 0.74 e	8.23 ± 0.16 f	Red
Zuosaner	2007	15.99 ± 0.09 a	27.67 ± 0.88 i	9.11 ± 0.06 a	16.71 ± 0.07 g	7.31 ± 0.69 ab	3.11 ± 0.53 a	15.41 ± 0.32 i	Red
Shuanghong	2007	16.49 ± 0.85 a	25.78 ± 0.54 h	9.56 ± 0.18 ab	17.12 ± 0.94 g	7.45 ± 0.64 ab	4.56 ± 0.75 d	7.82 ± 0.03 ef	Red
Shuangfen	2007	17.27 ± 0.07 ab	22.33 ± 0.64 e	10.11 ± 0.83 ab	12.40 ± 0.23 cd	8.02 ± 0.95 abc	3.11 ± 0.44 a	8.33 ± 0.04 f	Red
Shuangyou	2007	25.15 ± 1.02 d	17.00 ± 0.86 c	16.67 ± 0.84 cde	9.53 ± 0.21 b	13.48 ± 0.84 e	3.67 ± 0.66 b	3.67 ± 0.10 c	Red
Euro-Asian hybrids									
Zuohongyi	2007	19.54 ± 0.54 b	19.56 ± 0.57 d	11.22 ± 0.44 ab	12.05 ± 0.50 c	9.51 ± 0.72 cd	5.78 ± 0.65 g	3.08 ± 0.14 bc	Red
Zuoyouhong	2007	36.66 ± 0.49 f	14.55 ± 0.45 b	30.67 ± 1.25 f	9.43 ± 0.11 b	25.97 ± 1.38 g	6.11 ± 0.43 e	1.37 ± 0.04 a	Red
NW196	2007	31.65 ± 0.98 e	24.01 ± 0.97 fg	30.45 ± 0.88 f	18.36 ± 0.91 h	27.62 ± 1.01 h	4.11 ± 0.66 c	15.85 ± 0.34 i	Red
NW196	2008	42.64 ± 0.52 g	16.62 ± 0.44 c	41.41 ± 1.23 h	12.17 ± 0.52 c	30.22 ± 1.07 i	4.72 ± 0.54 d	9.51 ± 0.54 d	Red
Euro-American hybrids									
Kyoho	2007	50.74 ± 1.14 h	17.60 ± 0.47 c	45.60 ± 1.53 I	14.21 ± 0.46 e	40.01 ± 1.69 J	13.10 ± 0.91 n	1.57 ± 0.16 a	Red
V. vinifera L.									
Cabernet Sauvignon	2007	99.28 ± 2.14 k	25.24 ± 0.55 gh	95.80 ± 3.21 l	22.97 ± 0.36 J	93.33 ± 2.10 m	12.59 ± 0.81 m	9.07 ± 0.36 g	Red
Cabernet Sauvignon (pomace)	2007	84.78 ± 1.59 J	23.79 ± 0.68 f	74.31 ± 2.41 k	18.82 ± 0.64 h	61.26 ± 2.21 l	7.41 ± 0.74 k	2.54 ± 0.25 b	Red
Muscadines (V.rotundifolia Michx.)									
Noble	2008	51.79 ± 0.84 h	22.49 ± 0.43 e	28.61 ± 0.81 f	12.08 ± 0.14 c	19.67 ± 1.45 f	5.22 ± 0.61 f	11.63 ± 0.34 h	Red
Fry	2008	68.29 ± 0.24 I	12.11 ± 0.46 a	61.70 ± 1.41 J	6.46 ± 0.05 a	57.25 ± 2.24 k	5.55 ± 0.79 fg	ND	Bronze
Carlos	2008	52.69 ± 0.59 h	22.62 ± 0.38 e	37.61 ± 0.76 g	18.19 ± 0.77 h	34.22 ± 0.66 i	17.63 ± 0.86 p	ND	Bronze

ND: not determined.
[a] Values represent means of triplicate determination ± S.D.
[b] Data were analysed by ANOVA and within each column different letters indicate statistically different values according to post hoc comparison (Student Newman Keuls) at $p = 0.05$.

Trolox standard solutions were prepared at a concentration ranging from 100 to 2000 μM.

2.2.3.3. Determination of reducing power (FRAP). The FRAP assay was done according to Benzie and Strain (1996). The radical-scavenging activity of the test samples was expressed as trolox equivalent antioxidant capacity (μM TE/g DM). Trolox standard solutions were prepared at a concentration ranging from 100 to 1000 μM.

2.3. Statistical analyses

Experimental results were means ± S.D. of three parallel measurements. Data were subjected to ANOVA and differences among cultivars were tested by post hoc comparison test (Student Newman Keuls) at $p = 0.05$. The principal component analysis (PCA) (Jolliffe, 1986) was done to detect clustering formation and establish relations between samples and phenolic compound or antioxidant properties. Microsoft Excel 2003 and SPSS 16.0 for Windows were used for this analysis and Pearson's correlation coefficients calculation.

3. Results and discussion

3.1. Variation of phenolic compounds among the grape cultivars

The total phenolic compounds in grape seeds varied significantly among the grape cultivars studied (Table 1). *V. vinifera* "Cabernet Sauvignon" had the highest total phenolic contents in seeds, followed by Muscadine grapes, while the lowest appeared in the Oriental *Vitis* species. As expected, phenolic contents of the Euro-Asian or Euro-American hybrids fell between the parents. Similar distribution was found in the total flavanoid and total flavan-3-ols contents in seeds among the grape cultivars studied. This result was further illustrated by the PCA of the phenolic compound contents in grape seeds (Fig. 1a). Five different classes were well separated, with Class A including all the Oriental *Vitis* grapes, and Class B–E representing the Euro-Asian hybrids, Euro-American hybrid, Musadine and *V. vinifera* grapes, respectively. Musadine and *V. vinifera* (classes D and E) were clustered with high scores on PC1, while Oriental *Vitis* species (classes A) were clustered with negative PC1 scores. Furthermore, the PC1 scores of these five classes with a decreasing order of Class E > D > C > B > A. Additionally, a

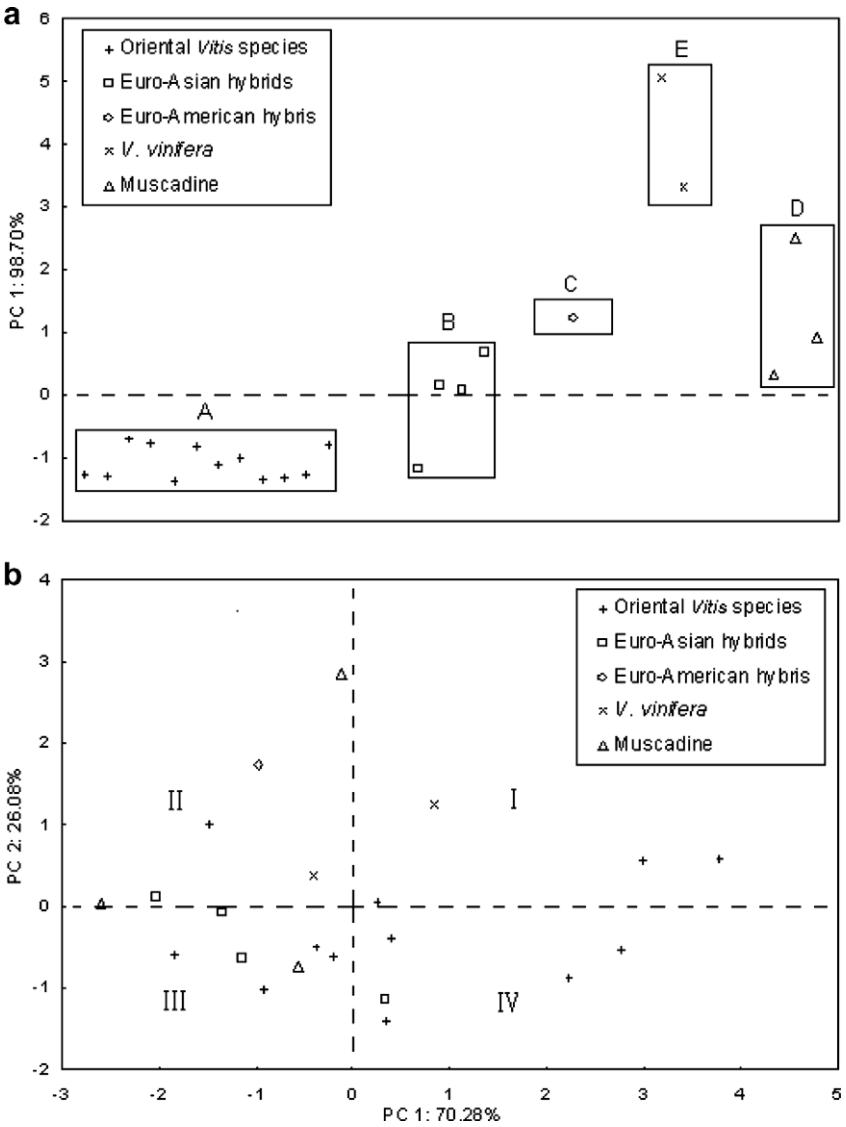

Fig. 1. Principal component score of the investigated grape variety seeds (a) and skins (b) according to PC1 and PC2 obtained by contents of phenolic compounds (total phenols, flavonoids, flavan-3-ols and (or) anthocyanins). Percentages represent the variance of each component.

significant variation of phenolic compounds in seeds was also found among different cultivars belonging to the same species (Table 1). For example, "Fry", a white table grape muscadine cultivar, had about a 2-fold higher total flavanoid and flavan-3-ols content than "Noble", a red wine muscadine cultivar.

Overall, less cultivar variation of phenolic compounds was found in the skins than in the seeds (Table 1). In contrast to the seeds, the total phenolic contents of skins were found higher among most cultivars of the Oriental *Vitis* species than the *V. vinifera* "Carbernet Sauvignon" and the muscadine grapes. For example, Oriental grapes *V. dividii* "Black Pearl" and *Vitis ficifolia* "Sangye" showed the highest values of the total phenols and flavanoids, respectively. For total flavan-3-ols content, Muscadine grape "Carlos" showed the highest value (17.63 mg PAE/g DM).

The skin anthocyanin contents varied among the oriental species/cultivars (Table 1). The highest value of total anthocyanin content was found in "Sangye" grape (averaging 21.48 mg MAE/g DM), which is about 2.5-fold higher than "Cabernet Sauvignon" (9.07 mg MAE/g DM). The lowest anthocyanin content was detected in Euro-Asian hybrid "Zuoyouhong" (1.37 mg MAE/g DM) and Euro-American hybrid "Kyoho" (1.57 mg MAE/g DM).

The principal component score of the skins in the product space failed to show clear differentiation of the five grape groups compared to the seeds (Fig. 1b). The classification based more on the

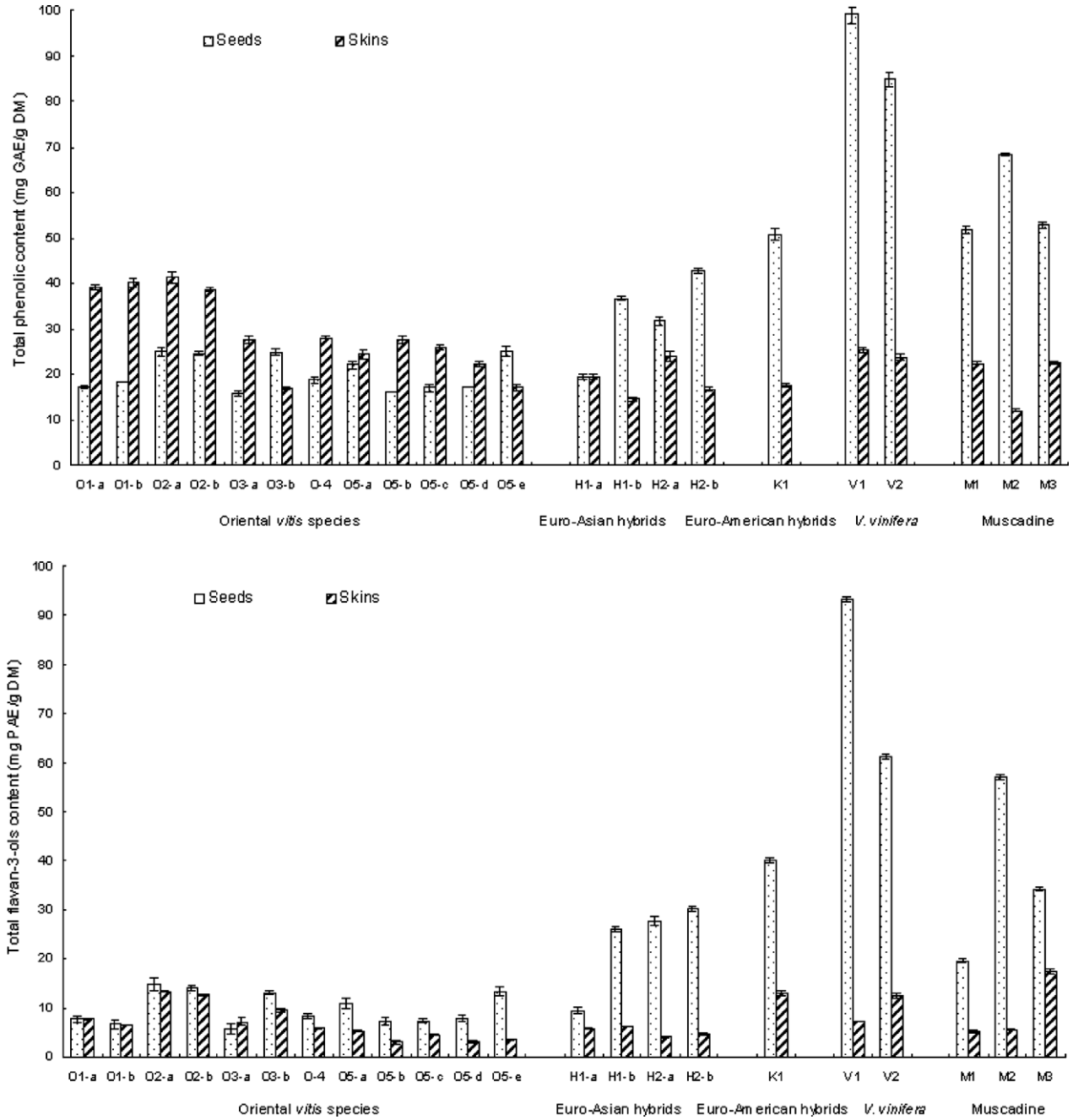

Fig. 2. Total phenolic contents (top) and flavan-3-ols contents (bottom) of seeds and skins in the grape varieties investigated. Data were expressed as mg GAE/g DM for phenols and mg PAE/g DM for flavan-3-ols. All assays were conducted in triplicate, and mean values were used. The vertical bars represented the standard deviation of each data point. Oriental *Vitis* species: (O1-a) Black Pearl, 2007; (O1-b) Black Pearl, 2008; (O2-a) Sangye, 2007; (O2-b) Sangye, 2008; (O3-a) Purple grape; (O3-b) White grape; (O4) Mao; (O5-a) Zuosanyi; (O5-b) Zuosaner; (O5-c) Shuanghong; (O5-d) Shuangfen; (O5-e) Shuangyou. Euro-Asian hybrids: (H1-a) Zuohongyi; (H1-b) Zuoyouhong; (H2-a) NW196, 2007; (H2-b) NW196, 2008. Euro-American hybrids: (K1) Kyoho. *V.vinifera* L.: (V1) Cabernet Sauvignon; (V2) Cabernet Sauvignon (pomace). Muscadines: (M1) Noble; (M2) Fry; (M3) Carlos.

specific cultivar rather than on the groups. The score for grape skins in the first two principal components explain 96.36% of the data matrix variance. PC1 represented 70.28% of the variance and it was correlated to total phenols, flavonoids and anthocyanins, whilst PC2 represented 26.08% of variance correlating to the total flavan-3-ols. The PC1 score (total phenols, flavonoids and anthocyanins) of Oriental grapes *V. dividii* "Black Pearl" and *V. ficifolia* "Sangye" were found to be much higher than *V. vinifera* and Muscadine as they had higher total phenols, flavonoids and anthocyanins in skin than others. In the PC2 (total flavan-3-ols), Muscadine grape "Carlos" and Euro-American hybrid "Kyoho" showed the highest score, indicating that they had higher total flavan-3-ols contents than other cultivars.

It was reported that the total phenols in skin extracts of *V. vinifera* and *V. rotundifolia* were lower than in seed extracts (Iacopini, Baldi, Storchi, & Sebastiani, 2008; Striegler et al., 2005). Similar results were also found in this study where the total phenols in seeds were much higher than in skins of the *V. vinifera* "Cabernet Sauvignon" and Muscadine "Fry" and "Noble" (Table 1). However, our study showed that the total phenols in most Oriental *Vitis* species skin extracts were higher than in seeds. Only *V. xunyangensis* "White Grape" and *V. amurensis* "Shuangyou" showed a different behavior, having a lower content of total phenols in skins than in seeds (Fig. 2).

The Musacdine grape "Fry", while possessing the second highest total phenolic content in seeds, had the lowest concentration of total phenol in skin among all the cultivars investigated. A similar phenomenon was also found in other cultivars (Fig. 2). There seemed to be a tendency that while the total phenol content is high in the seed, a relatively low figure was found in the skin, or vice versa. This is confirmed by a statistical analysis that there is a significant negative correlation (Pearson's correlation coefficient −0.509, significant at the 0.05 level) of total phenolic contents between seeds and skins among Oriental *Vitis* species, Euro-Asian hybrids and Muscadines. This result indicated that even the total phenolic contents are similar in the berries among different cultivars, distributions of phenolic compounds in seeds and skins varied greatly among them.

Although the flavan-3-ols were located in both grape skins and seeds, lower concentrations appeared in skins than in seeds, especially in *V. vinifera* "Cabernet Sauvignon" and Muscadine "Fry" (Fig. 2). Only the Oriental grape "Purple grape" showed a different behavior, having a higher level of flavan-3-ols in skins than in seeds. This finding had lead to a similar conclusion reported by Rodriguez Montealegre, Romero Peces, Chacon Vozmediano, Martinez Gascuena, and Garcia Romero (2006) in *V. vinifera* cultivars. The grape skins, in general, contain much lower concentrations of flavan-3-ols than seeds.

3.2. Antioxidant properties of grape extracts

The antioxidant activities found by different assays in seeds of investigated cultivars differed greatly (Table 2). *V. vinifera* "Cabernet Sauvignon" had the highest antioxidant values (422.18, 649.85

Table 2
Antioxidant activities of grape seed and skin extracts measured by DPPH, ABTS and FRAP methods.

Species/cultivars	Year	Seeds[a,b,c]			Skins[a,b,c]		
		DPPH	ABTS	FRAP	DPPH	ABTS	FRAP
Oriental Vitis species							
V. davidii (Roman.) Foex							
Black Pearl	2007	65.11 ± 1.14 cd	78.87 ± 2.54 a	84.47 ± 1.08 a	187.66 ± 5.47 i	368.67 ± 16.45 hi	239.18 ± 8.49 J
Black Pearl	2008	73.13 ± 1.54 de	110.14 ± 4.45 bcd	108.04 ± 5.21 bc	165.32 ± 6.64 h	328.67 ± 13.34 h	225.04 ± 14.26 ij
V. ficifolia Bunge.							
Sangye	2007	107.17 ± 2.59 g	164.22 ± 5.26 e	144.90 ± 2.56 d	275.96 ± 9.04 J	507.75 ± 15.92 J	312.04 ± 15.21 k
Sangye	2008	108.08 ± 1.85 g	174.78 ± 10.25 e	146.18 ± 3.33 d	247.66 ± 10.54 J	491.43 ± 19.89 J	297.18 ± 10.13 k
V.xunyangensis P. C. He							
Purple grape	2007	52.42 ± 0.98 a	76.33 ± 2.54 a	76.76 ± 1.52 a	136.58 ± 6.68 ef	168.21 ± 12.25 de	146.18 ± 4.89 cd
White grape	2008	101.78 ± 3.35 fg	168.87 ± 11.23 e	143.18 ± 4.23 d	118.51 ± 7.04 bcd	119.13 ± 5.18 b	135.92 ± 5.64 bc
V.quinquangularis Rehd.							
Mao grape	2007	79.35 ± 1.75 e	102.95 ± 5.68 bc	114.47 ± 5.19 b	133.66 ± 9.15 ef	213.21 ± 13.67 f	180.04 ± 5.97 fgh
V. amurensis Rupr.							
Zuosanyi	2007	77.25 ± 2.46 e	120.50 ± 4.59 dc	120.51 ± 4.32 c	152.96 ± 7.05 g	192.96 ± 10.25 ef	198.62 ± 12.14 h
Zuosaner	2007	56.42 ± 0.59 ab	88.11 ± 5.21 ab	81.02 ± 2.14 a	169.51 ± 6.31 h	259.62 ± 14.13 g	217.67 ± 7.15 i
Shuanghong	2007	59.42 ± 1.13 bc	94.68 ± 3.46 ab	86.22 ± 1.98 a	147.04 ± 4.94 f g	170.22 ± 6.97 de	198.14 ± 8.19 h
Shuangfen	2007	63.33 ± 1.08 bc	98.90 ± 1.18 abc	95.76 ± 6.64 ab	124.59 ± 5.04 cde	155.10 ± 5.64 cd	181.96 ± 10.04 fgh
Shuangyou	2007	95.17 ± 2.57 f	154.30 ± 13.14 e	140.98 ± 6.85 d	96.22 ± 4.36 a	97.50 ± 3.57 b	123.38 ± 5.48 ab
Euro-Asian hybrids							
Zuohongyi	2007	72.33 ± 0.94 de	126.13 ± 6.31 d	108.14 ± 5.23 bc	119.87 ± 4.95 bcd	112.68 ± 5.35 b	159.09 ± 8.21 de
Zuoyouhong	2007	164.83 ± 6.84 h	231.77 ± 9.54 f	221.01 ± 10.25 e	95.04 ± 2.67 a	71.76 ± 4.67 a	112.89 ± 5.55 a
NW196	2007	188.30 ± 5.91 i	215.62 ± 7.85 f	211.33 ± 12.15 e	136.60 ± 4.24 ef	221.46 ± 11.24 f	187.47 ± 4.69 gh
NW196	2008	228.00 ± 4.56 J	273.37 ± 14.25 g	287.61 ± 9.97 f	94.05 ± 1.85 a	108.21 ± 4.21 b	131.18 ± 9.64 bc
Euro-American hybrids							
Kyoho	2007	244.95 ± 8.54 k	349.43 ± 15.21 h	317.18 ± 11.58 g	138.73 ± 2.64 ef	170.49 ± 10.16 de	143.18 ± 8.5 bcd
V.vinifera L.							
Cabernet Sauvignon	2007	422.18 ± 9.26 o	649.85 ± 9.89 l	605.18 ± 19.57 l	132.34 ± 6.45 def	206.46 ± 14.86 f	165.47 ± 5.43 ef
Cabernet Sauvignon (pomace)	2007	324.62 ± 10.30 m	488.86 ± 12.14 J	449.18 ± 15.08 J	152.56 ± 4.87 g	258.21 ± 16.34 g	170.18 ± 3.39 efg
Muscadines (V.rotundifolia Michx.)							
Noble	2008	310.28 ± 6.65 l	438.16 ± 17.25 i	413.59 ± 9.48 i	156.81 ± 5.61 g	252.75 ± 6.65 g	227.61 ± 10.69 ij
Fry	2008	408.75 ± 11.52 n	620.69 ± 20.21 k	526.76 ± 14.14 k	110.01 ± 7.15 b	128.67 ± 7.23 bc	136.76 ± 5.84 bc
Carlos	2008	315.68 ± 7.59 l	441.96 ± 10.86 i	395.58 ± 11.15 h	196.18 ± 8.41 i	348.21 ± 18.65 h	241.76 ± 10.18 J

[a] Values represent means of triplicate determination ± S.D.
[b] Data were analysed by ANOVA and within each column different letters indicate statistically different values according to post hoc comparison (Student Newman Keuls) at $p = 0.05$.
[c] Results expressed as μM Trolox equivalents (μM TE/g DM).

and 605.18 μM TE/g DM for DPPH, ABTS and FRAP assay, respectively), followed by Muscadines, while the lowest appeared in the Oriental *Vitis* grapes. Similar to the phytochemical contents, the antioxidant activities of the Euro-Asian or Euro-American hybrids fell between the parents. The principal component analysis of the antioxidant activities in grape seeds also showed this tendency, with a decreasing order of *V. vinifera* > Musadine > Euro-American hybrids > Euro-Asian hybrids > Oriental *Vitis* species (Fig. 3a). The variation of antioxidant activities is closely correlated to the variation of total phenolic compounds in seeds, indicating that those cultivars richer in phenolic compounds also tend to have higher antiradical activities.

Antioxidant activities were found higher in skins among most of the Oriental *Vitis* species/cultivars than in *V. vinifera* "Carbernet Sauvignon" (Table 2). Scores of PC1 in the product space showed fewer differentiations of the five-class grapes (Fig. 3b). In general, fewer variables in antioxidant activities were found in the skins among the grape cultivars studied than in the seeds. For example, Oriental *Vitis* grapes "Sangye" had the highest antioxidant values (averaging 261.81, 499.59 and 304.61 μM TE/g DM for DPPH, ABTS and FRAP assay, respectively), while Euro-Asian hybrids "Zuoyouhong" had the lowest antioxidant values (95.04, 71.76 and 112.89 μM TE/g DM for DPPH, ABTS and FRAP assay, respectively).

Interestingly, in *V. vinifera* "Cabernet Sauvignon", Muscadines, Euro-Asian hybrids and Euro-American hybrids, all the three antioxidant values (DPPH, FRAP, and ABTS) of seeds were much higher than skins (Table 2), while in Oriental *Vitis* species/cultivars, these antioxidant values in seeds were mostly lower than in skins. The possible explanation could be due to the higher amount of polyphenolics such as anthocyanins in skins than seeds for Oriental *Vitis* species/cultivars. Slight differences among these antioxidant values may also be attributed to the multiple reaction characteristics and mechanisms of each method (Di Majo et al., 2005). Therefore, to accurately evaluate antioxidant properties of grape extracts, more antioxidant assays in both skins and seeds should be carried out.

Solvent and polarity may affect the single electron transfer (SET) and the hydrogen atom transfer (HAT), which are key aspects in the measurements of antioxidant capacity (Perez-Jimenez & Saura-Calixto, 2006). Antioxidant capacity values should therefore only be compared when the measurements are made with the same method and the effects of solvent should be tested first. In our experiment, for example, an acidic methanolic solvent was used for the extraction. However, the solvent could also contribute to the reduction of the radicals in both DPPH and ABTS assays, causing an overestimation of the antioxidant capacity of phenolic

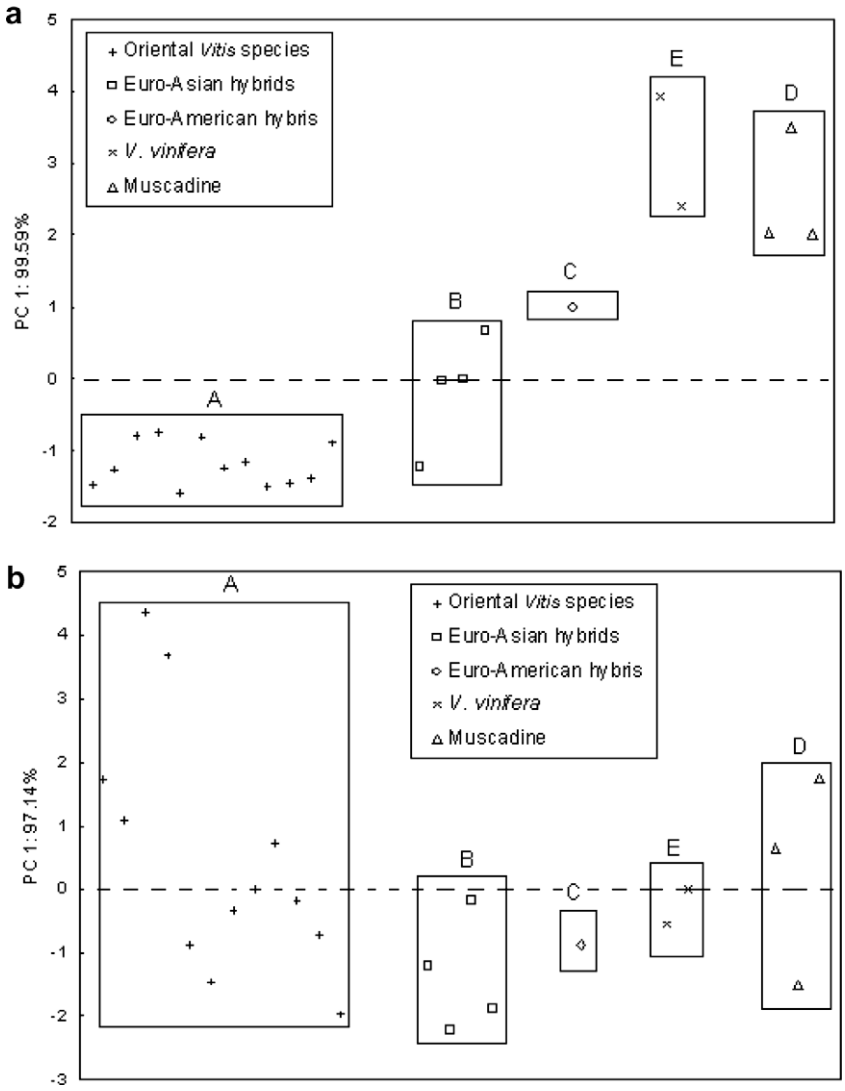

Fig. 3. Principal component score of the investigated grape variety seeds (a) and skins (b) according to PC1 obtained by contents of antioxidant properties (DPPH, ABTS, FRAP). Percentages represent the variance of PC1.

compounds. To eliminate this interference, the methods were modified by diluting the extracts with phosphate buffered saline (pH 7.4) in 1:4 for DPPH assay and 1:2 for ABTS assay. For FRAP assay, due to reactions in the acetate buffer (pH 3.6) system, little effect of solvent was found. Another important factor which was often neglected in the DPPH and ABTS assays was the establishment of a calibration curve. Due to the difference of initial maximum $DPPH^{\cdot}$ or $ABTS^{+}$ absorbances were obtained each time. A linearity eliminating calibration curve between the remaining $DPPH^{\cdot}$ or $ABTS^{+}$ absorbance after reaction and known solutions of Trolox may not accurately present the antioxidant capacity. Establishment of the calibration curve between percentage inhibition of radicals and known solutions of Trolox may therefore give a more proper evaluation.

3.3. Correlation between phenolic compounds and antioxidant capacity

A correlation analysis was done among the phenolic compounds and the antioxidant capacity parameters, as well as between each phenolic compound and antioxidant capacity measurement for all cultivars (Tables 3 and 4). Significant correlations among different antioxidant assays (DPPH, ABTS and FRAP) were found in both seeds and skins. This result suggests that these three assays are almost comparable and interchangeable in characterising the grape antioxidant capacities. These results are in agreement with other reports in the literature (Cimino, Sulfaro, Trombetta, Saija, & Tomaino, 2007; Li, Wang, Li, Li, & Wang, 2009). The total phenols (TP), total flavonoids (TFO) and total flavan-3-ols (TFL) contents of grape seeds exhibited a significant correlation ($p < 0.01$) with antioxidant properties with a decreasing order of TP > TFO > TFL (Table 3). The TP, TFO, TFL and TA (total anthocyanins) contents of grape skins also exhibited a significant correlation ($p < 0.01$) with antioxidant properties (TP > TFO > TA > TFL) (Table 4), with the only exception of TFL contents which showed no statistically significant correlation with the FRAP assay. The reason is probably because the flavan-3-ols are not the main phenolic compound in grape skins.

The significant correlations among the total phenols, total flavonoids and total flavan-3-ols in seeds strongly suggest that flavan-3-ols is the major compound of flavonoids, and flavonoids are the major compounds contributing to total phenols in grape seeds. The total phenols also exhibited a significant correlation ($r = 0.928$, $p < 0.01$) with total flavonoids and anthocyanins in skins ($r = 0.866$, $p < 0.01$) while it had no statistically significant correlation with the total flavan-3-ols (Table 4). Overall, a stronger correlation was found among these parameters in seeds than in skins. This may be attributed to more interferents such as reducing sugars, aromatic amine and amino acid in skins than in seeds.

Table 3
Pearson's correlation coefficients of antioxidant capacity (DPPH, ABTS, FRAP), total phenols (TP), total flavonoids (TFO) and total flavan-3-ols (TFL) in grape seeds.

	DPPH	ABTS	FRAP	TP	TFO	TFL
DPPH	1	0.991**	0.994**	0.949**	0.913**	0.901**
ABTS		1	0.996**	0.962**	0.922**	0.917**
FRAP			1	0.970**	0.934**	0.923**
TP				1	0.98**	0.968**
TFO					1	0.992**
TFL						1

** Correlation is significant at the 0.01 level (2-tailed).

Table 4
Pearson's correlation coefficients of antioxidant capacity (DPPH, ABTS, FRAP), total phenols (TP), total flavonoids (TFO), total flavan-3-ols (TFL) and total anthocyanins (TA) in grape skins.

	DPPH	ABTS	FRAP	TP	TFO	TFL	TA
DPPH	1	0.967**	0.956**	0.810**	0.825**	0.567**	0.669**
ABTS		1	0.948**	0.859**	0.865**	0.542**	0.732**
FRAP			1	0.830**	0.780**	0.405ns	0.757**
TP				1	0.928**	0.258ns	0.866**
TFO					1	0.488*	0.766**
TFL						1	−0.014ns
TA							1

ns: nonsignificant.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

4. Conclusions

Significant differences of total phenols and flavonoids were observed in both seeds and skins among the 18 grape cultivars studied. The total anthocyanin in skin was also significantly varied among these grapes. Among them, *V. vinifera* "Cabernet Sauvignon" and Muscadine grapes possess the most abundant phenolic compounds and antioxidant properties in seed, while the Oriental *Vitis* species "Black Pearl" and "Sangye" were found to be the richest in phenolic contents in skin. The oriental grapes had different distributing patterns of phenolic compounds and antioxidant properties from the non-oriental grapes, being higher in skins than in seeds in general. These findings are very important for future utilisation of the Oriental grape germplasm for improving nutritional properties of grape cultivars. A significantly negative correlation of phenolic compound distribution between seeds and skins was observed. Therefore, a more accurate assessment for the nutritional properties of different grape species/cultivars would require measuring both seeds and skins. This study has also proved that in general, the phenolic compounds and antioxidant properties in hybrids were intermediate between their parents.

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