Determination of Antioxidant Compounds and Antioxidant Activity of Six Table Grapes with Red Skin

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Abstract. Red grapes are a better source of antioxidants. Although studies have reported the major antioxidant contents and activities of grape cultivars, but the literature lacks information on detailed antioxidant composition and capacities of grape cultivars grown with rain-shelter-cultivation in Chengdu plain, south of China. In the present study, the total phenolics content (TPC), total flavonoids content (TFC), total flavanols content (TFAC), total anthocyanins content (TMAC) and antioxidant activity (DPPH, ABTS and FRAP) were detected and the results will provide basic knowledge about antioxidants of six red skin grapes. The results indicated the content rank order as follow: seeds > skin > pulp. Furthermore, ‘Red Globe’, ‘Jintianmeizhi’ and ‘Hongbaladuo’ have more antioxidants than other cultivars. Our results will provide a basic theory basis for consumers and further utilization of antioxidants.

1 Introduction

China, with about 15% of the world production in 2013, is the largest contributor to grape industry in the world (FAOSTAT 2013). In recently years, some southern regions of China, such as Chengdu plain, have planted lots of new grape cultivars and obtained more and more yield. Different from north of China, vine rows must be cultivated under the plastic rain shelter in order to obtain better commodity properties.

The composition and concentration of antioxidants in grapes may vary with variety, species, viticultural and environmental factors [1]. Kedage et al. investigated antioxidant activity of 11 grapes cultivars from Asian countries. Their results indicated that the differences in total phenol and total flavonoids content between the cultivars may depend on the grape colour [2]. Xu et al. have reported the phenolic compounds and antioxidant profiles of different Chinese grape cultivars, European grapes and Muscadine grapes [3]. Their study showed a significant difference of total phenols and flavonoids in both seeds and skins between these cultivars. Nile et al. explored the polyphenolic contents and the antioxidant activity of the skins and pulps of different grape cultivars. Their results suggest that the phenolic and flavonoid contents in extracts of grape skins and pulps showed statistically significant correlations with the free radical scavenging activity [4].

Although several studies reported the major antioxidant contents and activities of grape cultivars [2-4], but the literature lacks information on detailed antioxidant composition and capacities of grape cultivars

grown with rain-shelter-cultivation in Chengdu plain, south of China. In the present study, the results will provide basic knowledge about antioxidants of six red skin grapes.

2 Materials and methods

2.1 Plant material

Grape material (*Vitis vinifera* L.) cultivated with a bilateral cordon training system were collected on 2018, in a commercial table grape vineyard located in the territory of Yong-an town (N30°24', E103°59'), Chengdu, China. All the grape varieties were cultivated under the plastic rain shelter and the planting spacing was 3m × 1m. Field management (Fertilizer, pest control, and other vineyard operations) was conducted according to local practices.

Grape samples of 2kg for each variety were harvested manually at full mature stage. After that, grape berries were brought to the laboratory immediately. Seeds, skins and pulps were carefully separated manually and stored at -80°C.

2.2 Method

The TPC of seeds, skins and pulps extracts were determined by the Folin-Ciocalteu method [5]. The TFC of seeds, skins and pulps extracts was determined according to the method of Jia et al. [6]. The TFAC of

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seeds, skins and pulps extracts was detected with p-DMACA [7]. The TMAC of seed, skin and pulp extracts was estimated by the pH differential method [8]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was determined based on an assay modified by Brandwilliams et al. [9]. The ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) assay was based on the method from Re et al. [10]. The FRAP (ferric reducing antioxidant power) was measured by following a procedure derived from Benzie and Strain [11].

2.3 Statistical analysis

The results were presented as means $\pm$ SD and all analysis was performed in triplicate. SPSS 22.0 was used to analyse data by one way analysis of variance (ANOVA). A multiple comparison procedure of the treatment means was performed by Duncan Test. Significance of the differences was defined as $P < 0.05$.

3 Results

3.1. Cultivars characteristics

All tested grapes possess red appearance. Except seeds were not detected in 'Crimson', and all other cultivars have seeds.

Table 1. Cultivars characteristics

Grape cultivar	Skin color	Seeds (Y/N)
Red Globe	Red	Y
Jintianmeizhi	Red	Y
Manicure Finger	Red	Y
Crimson	Red	N
Hongbaladuo	Red	Y
Zhaopinghong	Red	Y

Y, seeds; N, no seeds.

3.2 Analysis of TPC in different tissues

As shown in Table 2, there were significant differences ($p < 0.05$) between the total content of phenolic compounds in different grape cultivars and tissues. The TPC in different grape skins ranged from 279.9 to 990.1 mg GAE/kg FW. 'Red Globe' contained the highest contents of total phenolics whereas the lowest values for that parameter were found in 'Crimson'. The TPC of pulp and of seeds ranged from 76.3 to 139.8 mg GAE/kg FW and from 2752.3 to 4062 mg GAE/kg FW respectively (Table 2). 'Jintianmeizhi' grape contained the highest TPC in the skins and pulps, 'Hongbaladuo' in seeds, whereas the lowest values were measured from 'Crimson'. The results showed that TPC in different tissues, seeds had highest content, followed by skins, while TPC in pulps was absolutely less than that of skin and seeds.

Table 2. TPC in different Cultivars and tissues

Grape cultivar	TPC (mg GAE/kg FW)		
	Skin	Pulp	Seed
Red Globe	456.3 $\pm$ 12.1c	138.6 $\pm$ 6.6a	3799.5 $\pm$ 124b
Jintianmeizhi	578.1 $\pm$ 11.4a	139.8 $\pm$ 15.0a	2877.4 $\pm$ 184c
Manicure Finger	496.2 $\pm$ 15.0b	114.8 $\pm$ 19.0b	2752.3 $\pm$ 212c
Crimson	279.9 $\pm$ 19.1e	76.3 $\pm$ 5.7c	ND
Hongbaladuo	420.7 $\pm$ 37.6d	139.5 $\pm$ 16.2a	4062 $\pm$ 160a
Zhaopinghong	288.6 $\pm$ 10.2e	127.2 $\pm$ 8.7ab	4006.3 $\pm$ 185a

ND, not detected; GAE, gallic acid equivalent.

Values are means $\pm$ SD values of three replicates.

Different letters indicate significant difference at $p < 0.05$ by Duncan's test.

3.3 Analysis of TFC in different tissues

TFC and TPC presented a similar rank order among grape tissues extracts, but some difference in different cultivars (Table 3). TFC of skin in different grape varieties ranged from 720.6 to 990.1 mg RE/kg FW, and 'Red Globe' contained the highest contents of total flavonoids. TFC in pulps ranged from 165.3 to 271.2 mg RE/kg FW, and 'Hongbaladuo' contained the highest contents of total flavonoids whereas the lowest values for that parameter were found in 'Crimson'. TFC in seeds ranged from 11153.7 to 13422.3 mg RE/kg FW, 'Red Globe' contained the highest. Among all cultivars, seeds had the highest TFC, followed by those from skins and pulps.

Table 3. TFC in different Cultivars and tissues

Grape cultivar	TFC (mg RE/kg FW)		
	Skin	Pulp	Seed
Red Globe	990.1 $\pm$ 61.5a	188.5 $\pm$ 21.7cd	13422.3 $\pm$ 549.2a
Jintianmeizhi	900.0 $\pm$ 30.2ab	201.9 $\pm$ 28.9c	11193.1 $\pm$ 270.4c
Manicure Finger	867.2 $\pm$ 66.0b	210.1 $\pm$ 29.1c	11153.7 $\pm$ 733.6c
Crimson	720.6 $\pm$ 34.6c	165.3 $\pm$ 4.0d	ND
Hongbaladuo	866.0 $\pm$ 67.1b	271.2 $\pm$ 15.2a	12793.4 $\pm$ 419.5b
Zhaopinghong	758.5 $\pm$ 56.3c	232.7 $\pm$ 18.3b	12999.1 $\pm$ 288.7b

ND, not detected; RE, gallic acid equivalent.

Values are means $\pm$ SD values of three replicates.

Different letters indicate significant difference at $p < 0.05$ by Duncan's test.

3.4 Analysis of TFAC in different tissues

TFAC of seeds, skins and pulps extracts measured by p-DMACA was shown in Table 4. Total flavanols content of peel ranged from 90.8 to 180.6 mg CE/kg FW (Table 4). Skin of 'Manicure Finger' contained the highest TFAC while the lowest was found in 'Zhaopinghong'. TFAC in seeds ranged from 1660.5 to 2340 mg CE/kg FW, and was the highest for 'Red Golbe' seeds extract (Table 4). Pulps extract TFAC was significantly lower than that of skins and seeds, pulps extract of 'Jintianmeizhi' was the highest (15.5 $\pm$ 0.2 mg CE/kg FW) and that of 'Crimson' was the lowest.

Table 4. TFAC in different Cultivars and tissues

Grape cultivar	TFAC (mg CE/kg FW)		
	Skin	Pulp	Seed
Red Globe	150.3±9.4bc	17.7±1.5ab	2340.0±120.4a
Jintianmeizhi	160.7±7.1b	18.8±1.1a	1690.1±130.7c
Manicure Finger	180.6±17.2a	14.4±1.3d	1660.5±30.3c
Crimson	140.9±11.3c	12.1±1.0e	ND
Hongbaladuo	120.3±7d	16.4±1.3bc	1980.8±80.4b
Zhaopinghong	90.8±8.2e	14.0±0.3d	1850.4±80.9b

ND, not detected; CE, catechin equivalent.

Values are means ± SD values of three replicates.

Different letters indicate significant difference at $p < 0.05$ by Duncan's test.

3.5 Analysis of TMAC in different tissues

The grape colour is a vital factor that consumer choose, while which was influenced by TMAC. TMAC of skin and pulp were high in red peel cultivars (from 325.4 to 2816.7 mg C3GE/kg FW), (Table 5). The TMAC was particularly low in 'Hongbaladuo' and 'Jintianmeizhi'. In pulps, the order of TMAC was different from that in skins, while the values were lower remarkably than skins. Moreover, TMAC of seeds in all cultivars were not detected because seeds may not contain anthocyanins.

Table 5. TMAC in different Cultivars and tissues

Grape cultivar	TMAC (mg C3GE/kg FW)		
	Skin	Pulp	Seed
Red Globe	2816.7±40.4a	22.8±1.1a	NA
Jintianmeizhi	325.4±10.3c	7.8±1.2b	NA
Manicure Finger	1209.6±62.4b	24.9±2.5a	NA
Crimson	1418.8±51.4b	20.8±3.4a	ND
Hongbaladuo	499.4±16.4c	20.2±4.5a	NA
Zhaopinghong	1241.1±84.4b	9.7±2.7b	NA

ND, not detected; NA, not available; and C3GE, cyanidin 3-glucoside equivalent.

Values are means ± SD values of three replicates.

Different letters indicate significant difference at $p < 0.05$ by Duncan's test.

3.6 Analysis of antioxidant activity in different tissues

Different cultivars and tissues' DPPH, ABTS and FRAP values were exhibited in Table 6, 7 and 8 on the basis of μmol Trolox equivalent (TE)/g FW.

In the skin, DPPH free radical scavenging capacity ranged from 23.2 to 30.2; ABTS ranged from 42.3 to 52.6; and by FRAP, from 879.1 to 1227.8. In the seeds, antioxidant activity of DPPH, ABTS and FRAP ranged from 94.8 to 118.3, from 173.9 to 200.5 and from 2804.5 to 4083.0, respectively. However, the DPPH, ABTS and FRAP values of pulp ranged separately from 15.2 to 20.3, 14.2 to 17.7 and from 190.4 to 269.9.

By comparing the antioxidant activity data of 6 red skin grape cultivars determined by DPPH, ABTS and FRAP assay, results suggested that seeds were the highest

in antioxidant activity followed by skins. Pulp fractions of grape showed the lowest antioxidant activity (Table 6, 7, 8). In the skin, the activity of DPPH and FRAP in 'Hongbaladuo' were higher than other three cultivars and in ABTS was 'Manicure Finger'. Nonetheless, the activity of DPPH, ABTS and FRAP in seeds and pulp had not significant correlation with skin colour in all tested cultivars. The results indicated that the seeds of tested grape cultivars were the best source of antioxidants content and activity, followed by skins and then pulps of grapes.

Table 6. DPPH in different Cultivars and tissues

Grape cultivar	DPPH ($\mu\text{mol TE/g FW}$)		
	Skin	Pulp	Seed
Red Globe	24.0±1.2d	20.3±1.1a	111.4±6.1b
Jintianmeizhi	28.4±2.1b	19.7±1.4a	96.7±4.2c
Manicure Finger	28.6±1.7b	18.2±0.9ab	94.8±2.8c
Crimson	26.0±0.5c	15.2±1.5c	ND
Hongbaladuo	30.2±0.8a	19.0±0.5ab	112.6±2.7b
Zhaopinghong	23.2±1.4d	18.6±1.7ab	118.3±3.8a

ND, not detected; TE, trolox equivalent.

Values are means ± SD values of three replicates.

Different letters indicate significant difference at $p < 0.05$ by Duncan's test.

Table 7. ABTS in different Cultivars and tissues

Grape cultivar	ABTS ($\mu\text{mol TE/g FW}$)		
	Skin	Skin	Skin
Red Globe	46.6±1.0b	17.7±0.5a	192.5±9.5b
Jintianmeizhi	47.9±2.0b	17.3±0.6a	189.9±3.9bc
Manicure Finger	52.6±1.8a	16.8±0.5ab	173.9±12.2c
Crimson	42.3±1.4c	14.2±1.0b	ND
Hongbaladuo	44.7±3.2bc	17.3±0.8a	193.7±8.4b
Zhaopinghong	46.6±3.2b	16.6±1.1ab	200.5±7.8a

ND, not detected; TE, trolox equivalent.

Values are means ± SD values of three replicates.

Different letters indicate significant difference at $p < 0.05$ by Duncan's test.

Table 8. FRAP Sin different Cultivars and tissues

Grape cultivar	FRAP ($\mu\text{mol TE/g FW}$)		
	Skin	Skin	Skin
Red Globe	933.5±67.7c	247.9±8.3b	4083.0±364.9a
Jintianmeizhi	946.9±82.1c	266.6±16.9a	2840.5±213.1c
Manicure Finger	879.1±56.3d	257.8±9.1ab	2804.5±361.8c
Crimson	1003.0±52.7b	190.4±14.1c	ND
Hongbaladuo	1227.8±41.4a	269.9±12.6a	3830.6±385.2b
Zhaopinghong	914.0±19.9c	247.6±8.6b	3898.6±449.8b

ND, not detected; TE, trolox equivalent.

Values are means ± SD values of three replicates.

Different letters indicate significant difference at $p < 0.05$ by Duncan's test.

4 Discussion

Yilmaz et al. observed that the TPC of the pulps of grape varieties were remarkably lower than those of seeds and skins[12]. Farhadi et al. discovered 'Ghara Shani' grape skin contain the highest content of total phenolic and anthocyanin and cane of 'Ghara Shani' grape contains the highest amount of flavonoid in five native grape cultivars in West Azerbaijan province, Iran[13]. In this study, a large amount of different antioxidants are present in the skins, pulps and seeds of grapes. The highest TPC, TFC and TFAC were determined in the seeds of grapes. The skins of grapes are another better source of TPC, TFC, TFAC and TMAC than pulp. Significant differences in TPC, TFAC and TFC between grapes are attributed to the localization of phenolic compounds mainly in the skin and seeds of grapes. Therefore, above results indicated various cultivars and tissues of grapevine are major factors for composition and content of antioxidants.

The antioxidant activity of different grapes detected by DPPH, ABTS or FRAP have been studied widely in the literature. DPPH and ABTS were used to investigate the antioxidant capacities among four grapes pomace extracts [14]. In present study, DPPH, ABTS and FRAP were employed to evaluate the antioxidant activity in different grape varieties. The results showed seeds were the highest in antioxidant activity followed by skins. Pulp fractions of grape showed the lowest antioxidant activity.

5 Conclusion

Red grape is a better source of antioxidants, and the content rank order as follow: seeds > skin > pulp. Furthermore, 'Red Globe', 'Jintianmeizhi' and 'Hongbaladuo' have more antioxidants than other cultivars. Our results will provide a basic theory basis for consumers and further utilization of antioxidants.

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