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...
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± 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       | Red        | Y           |
| 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±12.1c        | 138.6±6.6a         | 3799.5±124b       |
| Jintianmeizhi  | 578.1±11.4a        | 139.8±15.0a        | 2877.4±184c       |
| Manicure       | 496.2±15.0b        | 114.8±19.0b        | 2752.3±212c       |
| Finger         | 279.9±19.1c        | 76.3±5.7c          | ND                |
| Crimson        | 420.7±37.6d        | 139.5±16.2a        | 4062±160a         |
| Hongbaladuo    | 288.6±10.2c        | 127.2±8.7ab        | 4006.3±185a       |

ND, not detected; GAE, gallic acid equivalent. Values are means ± 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±61.5a       | 188.5±21.7cd      | 13422.3±549.2a    |
| Jintianmeizhi  | 900.0±30.2ab      | 201.9±28.9c       | 11993.1±270.4c    |
| Manicure       | 867.2±66.0b       | 210.1±29.1c       | 11153.7±733.6c    |
| Finger         | 720.6±34.6c       | 165.3±4.0d        | ND                |
| Crimson        | 866.0±67.1b       | 271.2±15.2a       | 12793.4±419.5b    |
| Hongbaladuo    | 785.8±56.3c       | 232.7±18.3b       | 12999.1±288.7b    |

ND, not detected; RE, gallic acid equivalent. Values are means ± 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 from1660.5 to 2340 mg CE/kg FW, and was the highest for ‘Red Globe’ 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 ±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          | 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       | 1241.8±84.5b       | 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.

3.5 Analysis of TMAC in different tissues

The grape colour is a vital factor that consumer chooses, 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 ‘Hongbaladou’ 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       | 1241.8±84.5b         | 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’ DPHH, 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, DPHH free radical scavenging capacity ranged from 23.2 to 30.2; ABTS ranged from 42.3 to 52.6; and by FRAP, from 670.1 to 1227.8. In the seeds, antioxidant activity of DPHH, ABTS and FRAP ranged from 94.8 to 173.9. From 200.5 and from 2804.5 to 4083.0, respectively. However, the DPHH, 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 DPHH, 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 DPHH, 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 (μmol 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±0.9d           | 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 (μmol TE/g FW) | Skin | Skin | Skin |
|--------------------|---------------------|------|------|------|
| Red Globe          | 46.6±1.0b           | 17.3±0.5a | 192.5±9.5b |
| Jintianmeizhi      | 47.9±2.0b           | 17.3±0.6a | 189.9±3.9c |
| Manicure Finger    | 52.6±1.8a           | 16.8±0.5ab | 173.7±12.2c |
| Crimson            | 42.3±2.1c           | 14.2±1.0ab | ND |
| Hongbaladuo        | 44.7±3.2c           | 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 (μmol 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.

References

1. J. Yang, TE. Martinson, RH. Liu. Phytochemical profiles and antioxidant activities of wine grapes. Food Chem 116:332-339(2009).
2. V.V. Kedage, V.V. Tilak, G.B. Dixit, T.P.A. Devasagayam, M. Mhatre. A study of antioxidant properties of some varieties of grapes (Vitis vinifera L.). Crit Rev Food Sci Nutri 47:175-185(2007).
3. C. Xu, Y. Zhang, L. Cao, J. Lu. Phenolic compounds and antioxidant properties of different grape cultivars grown in China. Food Chem 119:1557-1565(2010).
4. S.H. Nile, S.H. Kim, E.Y. Ko, S.W. Park. Polyphenolic Contents and Antioxidant Properties of Different Grape (V. vinifera, V. labrusca, and V. hybrid) Cultivars. BioMed Res. Int., 5 (2013).
5. V.L. Singleton, J.A. Rossi. Colorimetry of total phenols with phosphomolybdic phosphotungstic acid reagents. Am J Enol Vitic 16: 144-158(1965).
6. Z.S. Jia, M.C. Tang, J.M. Wu. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64(4): 555-559(1999).
7. Y.G. Li, G. Tanner, P. Larkin. The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. J Sci Food Agric 70(1): 89-101(1996).
8. X.Q. Wang, C.Y. Li, D. Liang, Y.J. Zou, P.M. Li, F. Ma. Phenolic compounds and antioxidant activity in red-fleshed apples. J Funct Foods 18: 1086-1094(2015).
9. W. Brandwilliams, M.E. Cuvelier, M.E. Berset. Use of a free-radical method to evaluate antioxidant activity. Food Sci Technol 28(1):25-30 (1995).
10. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 26(9-10): 1231-1237(1999).
11. I.F.F. Benzie, J.J. Strain. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. Anal Biochem 239(1): 70-76(1996).
12. Y. Yilmaz, Z. Göksel, S.S. Erdoğan, A. Öztürk, A. Atak, Ç. Özer. Antioxidant activity and phenolic content of seed, skin and pulp parts of 22 grape (Vitis vinifera L.) cultivars (4 common and 18 registered or candidate for registration). J Food Proces Preser 39: 1682-1691(2015).
13. K. Farhadi, F. Esmaeilzadeh, M. Hatami, M. Forough, R. Molaie. Determination of phenolic compounds content and antioxidant activity in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran. Food Chem 199: 847-855(2016).
14. Y.X. Xu, S. Burton, C. Kim, E. Sismour. Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties. Food Sci Nutr 4(1):125-133 (2016).