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Phenolic content and antioxidant activity of wine grapes and table grapes

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The polyphenols and total antioxidant activities of four wine grapes (Cabernet Sauvignon, Cabenet Franc, Merlot, Cabernet Gernischt) and four table grapes (Muscat, Red Globe, Vitis labruscana Kyoho, Milk grape) were determined and compared. The concentrations of phenolic, flavonoid, anthocyanins and resveratrol of 8 grape varieties were examined. The antioxidant activity including diphenyl picrylhydrazyl radical (DPPH-) scavenging, azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing power was evaluated. The results indicated that cabernet gernischt contained the highest total phenolic content, total flavonoid content and total anthocyanin content with 257.0 \pm 8.9, 607.7 \pm 24.3 and 164.2 \pm 6.1 mg/100 g, respectively. Merlot had the highest resveratrol content with 11.7 \pm 0.61 µg/100 g of fresh sample. The table grapes have less total phenolic, total flavonoid and total anthocyanin content. Cabernet sauvignon, cabernet gernischt and merlot possessed higher antioxidant capacity. On the contrary, the table grapes have less antioxidant capacity. Phenolic content of these fruits is significantly correlated with antioxidant capacity. These findings revealed that phytochemicals in the selected grapes have potent antioxidant activities.

Key words: Grape, phenolic, flavonoid, anthocyanin, antioxidant activity.

INTRODUCTION

A grape is a non-climacteric fruit that grows on the perennial and deciduous woody vines of the genus Vitis. There are about 60 species of Vitis, which are mainly found in the temperate zones of the northern hemisphere and almost equally distributed between America and Asia (Mullins et al., 1992). Grapes contain large amounts of phytochemicals including phenolics, flavonoids, anthocyanins and resveratrol, which offer health benefits. Antioxidant compounds include vitamins, phenols, carotenoids, and flavonoids. Among the last group, flavones. isoflavones, flavonones, flavonols,

anthocyanins and catechins are the most important, and exhibit substantial antioxidant activity (Gao et al., 1997; Wang et al., 1997). Grapes are rich in phenolics, flavonoids, and anthocyanins, which have been suggested to be responsible for their health benefits (Yang et al., 2009). Grape phenolics, especially high in the grape peel, are classified into two groups: the flavonoids and non-flavonoids. The flavonoids include flavonols flavan-3-ols (catechin), (quercetin) and anthocvanins. The non-flavonoids encompass hydroxybenzoates (gallic acid), hydroxycinnamates and stilbenes (resveratrol). Besides antioxidant activity, flavonoids have many biological activities such as the inhibition of plasma platelet aggregation and cyclooxygenase activity, the suppression of histamine release and SRS-A biosynthesis in vitro, potent nitric

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oxide radical scavenging activity and exhibiting antibacterial, antiviral, anti-inflammatory and antiallergenic effects (Cook and Samman, 1996). Anthocyanins are pigments, which are natural responsible for the orange, red, blue, violet colours of some fruits and flowers. Beside the attractive colour their positive health effects are also significant (Kong et al., 2003).

Grapes are one of the major dietary sources of anthocyanins, which are responsible for the colouring of black, red and purple grapes. Anthocyanins are reported to have antioxidant activity, anti-inflammatory activity, anticancer activity, apoptotic induction effect, α -glucosidase inhibition activity, vision benefits and effects on collagen, blood platelet aggregation and capillary permeability and fragility (Hou et al., 2003).

There are few research papers on the phenolic content and antioxidant activity of wine grapes and table grapes. Yang et al. (2009) represented some of the reports on the phytochemical profiles and antioxidant activities of wine grapes. Hogan et al. (2009) reported on the antioxidant properties of Norton (Vitis aestivalis) and Cabernet Franc (Vitis vinifera) wine grapes. Breksa et al. (2010) represented the reports on the antioxidant activity and phenolic content of 16 raisin grape (Vitis vinifera L.) cultivars and selections. Mulero et al. (2010) reported on the antioxidant activity and phenolic composition of organic and conventional grapes and wines. Studies from our laboratory represent one of the few attempts to compare the phenolic content and antioxidant capacity of wine grapes with table grapes. The objectives for this study were to determine the profiles of total phenolics, total flavonoids, total anthocyanins and resveratrol in wine grapes and table grapes, and the antioxidant capacity of eight grape cultivars.

MATERIALS AND METHODS

Gallic acid, rutin and resveratrol were purchased from National Institutes for Food and Drug Control. Acetone, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, potassium chloride were analytical grade from China. 2, 2`-azinobis-(3ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from BIO BASIC INC. (Buffalo, NY, USA). 2, 4, 6-Tri(2pyridyl)-1,3,5-triazine (DPTZ) was purchased from TCI Development Co., Ltd. (Shanghai, China). Folin-Ciocalteu reagent was obtained from Shanghai Yixin Biotechnology Co., Ltd.

Eight grape varieties including four wine grapes (Cabernet Sauvignon, Cabenet Franc, Merlot, Cabernet Gernischt) and four table grapes (Muscat, Red Globe, Vitis labruscana Kyoho, Milk grape) were obtained by grapeyard located in Changli, Hebei Province, China.

General descriptions of the grape varieties are given in Table 1. The grapes were harvested on ripening in the 2010 vintages. Grapes free from visible blemish or disease were selected. Three separate batches of grapes from different sites were used to prepare triplicate samples. For quantitative analysis, 50 to 70 grape berries, randomly selected from each grape variety, were collected for extraction. All data collected for each grape variety were reported as mean \pm SD for at least three replications.

Preparation of extracts

Total phenolics were extracted from fresh grapes by the modified method described by Yang et al. (2009). Briefly, 100 g of grapes were blended for 1 min in 100 g of 80% acetone using Waring blender with medium speed to remove seeds. After removal of the seeds and adding an additional 100 g of 80% acetone, the grapes were blended for 3 min using a Waring blender with high speed. The mixture was then homogenized in a High Speed Homogeniser for 3 min and filtered with vacuum under an ice bath. The acetone in the filtrate was evaporated using a rotary evaporator at 45°C until the weight of the evaporated filtrate was less than 10% of the weight of the original filtrate. All extracts were stored at -40°C until use. All extractions were performed in triplicate.

Estimation of total phenolic content

The total phenolic content in the grapes was determined using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999), which was modified by Yang et al. (2004). All extracts were diluted 1:10 with distilled water to obtain readings within the standard curve ranges of 0.0 to $600.0 \ \mu g$ of gallic acid/ml. The grape extracts were oxidised by the Folin-Ciocalteu reagent and the reaction was neutralised with sodium carbonate. The absorbance was measured at 760 nm after 90 min at room temperature by a SHIMADZU UV-2201 spectrophotometer. The absorbance values were then compared with those of standards with known gallic acid concentrations. All values were expressed as the mean (milligrams of gallic acid equivalents per 100 g of fresh sample) \pm SD for three replications.

Estimation of total flavonoid content

The total flavonoid content of the grape extract was determined using a modified colourimetric method (Jia et al., 1999). 0.25 ml of 1:10 diluted grape extracts was mixed with 1.25 ml of distilled water and subsequently with 0.075 ml of 5% sodium nitrite solution, and was allowed to react for 5 min. Then, a 0.15 ml of 10% aluminium chloride was added and allowed to further react for 6 min before 0.5 ml of 1 M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 3 ml. The absorbance of the mixture was immediately measured at a 510 nm wavelength against a prepared blank using a SHIMADZU UV-2201 spectrophotometer. The flavonoid content was determined by a rutin standard curve and expressed as the mean (milligrams of rutin equivalents per 100 g of fresh sample) \pm SD for three replications.

Estimation of total anthocyanin content

The monomeric anthocyanin content of the grape extract was measured using a modified pH differential method (Yang et al., 2009). The grape extracts were mixed thoroughly with 0.025 M potassium chloride buffer pH 1 in 1:2 ratio of extract to buffer. The grape extracts were then mixed similarly with a sodium acetate buffer pH 4.5. A SHIMADZU UV-2201 spectrophotometer was used to measure absorbance at 510 and 700 nm against a buffer blank at pH 1.0 and 4.5. Absorbance readings were converted to total milligrams of cyanidin 3-glucoside (C3G). The anthocyanin content was calculated as follows:

Total monomeric anthocyanins (mg/100 g) = $\triangle A \times MW \times 1000/(\epsilon \times C)$

 $\Delta A = (A_{510} - A_{700})_{Ph \ 1.0} - (A_{510} - A_{700})_{Ph \ 4.5}$

Where A is absorbance, MW (449.2) is molecular weight for C3G, ɛ

(26,900) is the molar absorptivity C3G and C is the concentration of the grape extract in milligrams per millilitre. The anthocyanin content was expressed as milligrams of C3G equivalents per 100 g of fresh grape for the triplicate extracts.

Reverse-phase HPLC analysis of resveratrol

A 3-ml grape sample was extracted in a test tube with 5 ml of ethyl ether, and then the mixture was put into a shaker with 200 rpm for 15 min. The organic phase was transferred into a new test tube. The residues were extracted with 5 ml of ethyl acetate twice using the same conditions. The organic solvent in the new test tube was evaporated by flushing with N₂. The dry residue was dissolved in 1 ml methanol, and the aliquots were then analysed by RP-HPLC.

Stock solution containing 14.4 mg/ml of resveratrol in methanol was prepared. The solution was stored at -4°C in the dark after elimination of oxygen with N2 to avoid the oxidation or decomposition of the phenolic compounds. Resveratrol in the grape extracts was quantified using a RP-HPLC procedure employing a Eclipse XDB-C18, 150 mm × 4.6 mm and 5 µm column. Samples of 20 µl standard or grape methanolic extracts were directly injected into the column. Elution was carried out with a mobile phase delivered using a Agilent HPLC pump at a flow rate of 1.2 ml/min according to the following gradient: the initial mixture was acetonitrile-water (9:91) adjusted to pH 2 with triflouroactic acid for 10 min; linear gradient to (25:75) in 10 min, hold for 10 min; linear gradient to (70:30) in 1 min, hold 12 min. An Agilent 1200 DAD detector was used for UV detection of analytes at 307 nm. Data signals were acquired and processed on a PC running the Agilent HPLC chemstation H4033A. Three HPLC injections were performed for each extract; peak heights were used for all calculations. The recoveries for resveratrol analyses were $104.28 \pm 2.71\%$ (n = 5).

The DPPH method

A standard solution of DPPH 24 mg/L was prepared in methanol. The methodology was based on previous reports (Katalinic et al., 2006). Of undiluted sample, 0.05 ml was mixed with 2 ml of DPPH solution, and the absorbance was measured immediately at 517 nm against a methanol blank. After 16 min at room temperature, the absorbance was read again.

The % inhibition of DPPH radical caused by a fruits sample was determined according to the following equation: $[(A_{C(0)}-A_{A(t)})/A_{C(0)}] \times 100$, where $A_{C(0)}$ is the absorbance of the sample at t = 0 min and $A_{A(t)}$ is the absorbance of sample at t = 16 min. Experiments were carried out in triplicate and results were expressed as mean values \pm SD. All experimental data showed a linear correlation to the amount of the antioxidants (straight line resulting from the fit by linear regression).

The ABTS method

Experiments were performed combining the experimental methodology of one paper (Herraiz and Galisteo, 2004) and is based on the oxidation of the ABTS by potassium persulfate to form a radical cation ABTS⁺⁺. ABTS was dissolved in water to prepare ABTS stock solution (7 mM). ABTS radical cation (ABTS⁺⁺) was produced by adding 2.45 mM potassium persulfate (final concentration). Diluted ABTS⁺⁺ solution with an absorbance of 0.70 \pm 0.02 at 734 nm was used as working solution. Absorbance readings (734 nm) were taken at 30°C exactly 5 min after initial mixing of 1 ml of diluted ABTS⁺⁺ solution and 10 µl of sample solution. UV-visible spectrophotometer was used to measure absorbances. Antioxidant activity (AA) was expressed as percentage inhibition of ABTS radical by using following equation:

$AA = 100 - [100 \times (A_{sample}/A_{control})]$

where A_{sample} is the absorbance of the sample at t = 5 min, and A_{control} is the absorbance of the control.

The FRAP assay

The FRAP (ferric reducing/antioxidant power) was performed on a modified version of the method by Valavanidis et al. (2009). It is based on the reducing power of antioxidants, which will reduce the Fe³⁺ to Fe²⁺ in the form of a blue complex (Fe²⁺/TPTZ). The reagent FRAP was prepared freshly every day. The FRAP value were calculated in µmol ascorbic acid equivalent per 100 g of fresh weight (f.w) extracts, instead of µmol kg⁻¹ of extract which was used by Benzie. The antioxidant capacity was calculated in comparison with ascorbic acid aqueous solutions, tested at five different concentrations (100 to 1000 µmol). The 500 µmol concentration of ascorbic acid is equivalent to 1000 FRAP values.

Statistical analysis

Evaluation and analysis of data were performed by means of the following software packages: SPSS for Windows software version 11.5 (SPSS Inc, USA). Results were subjected to ANOVA, and differences between means were located using Tukey's multiple comparison test. Correlations between various parameters were also investigated. Significance was determined at p < 0.05. All data were reported as the mean \pm SD of three replications.

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic content of 8 grape extracts were measured (Table 1). Cabernet Gernischt presented the highest total phenolic content (257.0 ± 8.9 mg of gallic acid equivalents/100 g of grape), followed by Cabernet Sauvignon, Merlot, Muscat, Cabenet Franc, Vitis labruscana Kyoho, Red Globe and Milk grape. The total phenolic content of Cabernet Gernischt, Cabernet Sauvignon, Merlot, Muscat and Milk grape was significantly different from each other (p < 0.05). However, significant differences in total phenolic content were not found among Muscat, Cabenet Franc, Red Globe and Vitis labruscana Kyoho, or among Milk grape, Cabenet Franc, Red Globe and Vitis labruscana Kyoho (p > 0.05). The results indicate that the wine grapes except Cabenet Franc contain high concentrations of total phenolics. In contrast, the table grapes have less phenolic content. An approximately 2.7-fold difference in total phenolic content was found between the highest and lowest ranked varieties, Cabernet Gernischt and Milk grape. Both genetic and agronomic or environmental factor play important roles in phenolic composition and concentration. It is well known that the composition of phenolics in grapes vary with variety, species, season, and environmental and management factors such as soil conditions, climate and crop load. As usual, the total phenolic of red grape skins is greatly higher than that of

Cultivar	Colour	Total phenolics (mg/100 g)	Total flavonoids (mg/100 g)	Total anthocyanin (mg/100 g)
Cabernet Sauvignon	Dark purple	219.5 ± 24.3^{b}	519.2 ± 15.6 ^a	$67.8 \pm 4.6^{\circ}$
Cabenet Franc	Dark purple	128.3 ± 19.6 ^{de}	303.5 ± 31.9 ^{bc}	$17.4 \pm 0.8^{\circ}$
Merlot	Dark purple	179.1 ± 13.3 ^c	396.2 ± 10.2 ^b	68.9 ± 3.5^{b}
Cabernet Gernischt	Dark purple	257.0 ± 8.9^{a}	607.7 ± 24.3^{a}	164.2 ± 6.1^{a}
Muscat	Red, purple	132.1 ± 11.2 ^d	306.5 ± 22.5 ^{bc}	13.7 ± 1.3 ^c
Red Globe	Red	115.8 ± 12.8 ^{de}	300.0 ± 1.4^{cd}	5.0 ± 0.3^{d}
Vitis labruscana Kyoho	Red, purple	127.4 ± 7.3 ^{de}	338.6 ± 34.6 ^{bc}	8.4 ± 0.4^{d}
Milk grape	Green	103.1 ± 21.3 ^e	228.7 ± 5.8^{d}	0.3 ± 0.08^{e}

Table 1. The total phenolic, total flavonoid, and total anthocyanin contents in 8 grape varieties.

^{a-e}Bar with no letters in common are significantly different (p < 0.05) in the same column.

white grapes due to the loss of the ability to produce anthocyanins in the skins of white grapes. Our results showed that the phenolic content of different grapes depends mainly on the grape skin colour. For instance, Milk grape with green skin had the lowest total phenolic content.

Total flavonoid content

Total flavonoids contents of 8 grapes are presented in Table 1. Among all the grape varieties analysed, Cabernet Gernischt had the highest total flavonoid content (607.7 ± 24.3 mg of rutin equivalents/100 g of fresh grapes), followed by Cabernet Sauvignon, Merlot, Vitis labruscana Kyoho, Muscat, Cabernet Franc, Red Globe and Milk grape. There was significant difference (p < 0.05) in total flavonoids content between Cabernet Gernischt, Merlot and Milk grape. However, significant differences in total flavonoids content were not observed between Cabernet Gernischt and Cabernet Sauvignon, between Milk grape and Red Globe or among Merlot, Cabenet Franc, Muscat and Vitis labruscana Kyoho(p >0.05). In this study, there was a 2.5-fold difference in total flavonoid content between the highest and lowest ranked varieties, Cabernet Gernischt and Milk grape.

Total anthocyanin content

Total anthocyanin contents of eight grape extracts were determined (Table 1). Cabernet Gernischt had the highest total anthocyanin content (164.2 ± 6.1 mg of cyanidin 3-glucoside equivalents/100 g of grapes), followed by Merlot, Cabernet Sauvignon, Cabenet Franc, Muscat, Vitis labruscana Kyoho, Red Globe and Milk grape. Significant differences were found in total anthocyanin content in comparisons between Cabernet Gernischt and Merlot, Cabernet Sauvignon and Vitis labruscana Kyoho, Cabenet Franc and Red Globe, Cabernet Gernischt and Milk grape(p < 0.05); however, significant differences in

total anthocyanin content were not found between Red Globe and Vitis labruscana Kyoho, or among Cabernet Sauvignon, Cabernet Franc and Muscat (p > 0.05). It has been reported that anthocyanins were not detected in any of green grape varieties, for example, Chardonnay, Riesling, Cayuga White, Marechal Foch and Vidal Blanc (Yang et al., 2009). However, our results showed that the total anthocyanin content in milk grape was 0.3 ± 0.08 mg of cyanidin 3-glucoside equivalents/100 g of grapes. In this study, the wine grape varieties contain high concentrations of total anthocyanins. On the contrary, the table grapes have less anthocyanin content. Cabernet Gernischt variety contained the highest total anthocyanin content, whereas Milk grape had the lowest, indicating almost 540-fold difference. The concentrations of the anthocyanins in red grapes vary greatly with the variety, species, maturity, production area and climate. As a characteristic associated with the variety, the level of anthocyanins in grapes may serve as an estimate of the red pigments and be useful for the classification of grape varieties and of relevant wines. Consequently. anthocyanins have been proposed as chemical markers to differentiate grape varieties and red wines.

Resveratrol content

The resveratrol contents of 8 grape extracts were determined (Figure 1). The Merlot variety presented the highest resveratrol content (11.7 \pm 0.61 µg/100g of fresh sample), followed by Cabernet Sauvignon (4.5 \pm 0.04), Cabernet Gernischt (3.2 \pm 0.006), Cabernet Franc (2.4 \pm 0.04), Vitis labruscana Kyoho (1.15 \pm 0.12), Muscat (1.0 \pm 0.01), Milk grape (0.9 \pm 0.01), and Red globe (0.78 \pm 0.05). There were significant differences (p < 0.05) in resveratrol content among Merlot, Cabernet Sauvignon, Cabernet Franc, Cabernet Gernischt and Muscat. However, no significant differences in resveratrol content were found among Muscat, Vitis labruscana Kyoho, Milk grape and Red globe (p > 0.05). In recent years, it has been discovered that resveratrol has several biological



Figure 1. Resveratrol content of 8 grape varieties (mean \pm SD, n = 3), bars with no letters in common are significantly different (p < 0.05)

effects, including anticancer activity for certain cancer types, cardio protection activity, antioxidant activity and inhibition of platelet aggregation, as well as antiinflammatory activity. There is increasing interest in resveratrol research owing to its pharmacological activity. Resveratrol is synthesized particularly in the leaf epidermis and the skin of grapes, and only trace amounts are present in the fruit flesh. Red wine generally contains higher amounts of trans-resveratrol than white wine. This is presumably due to the longer extraction time during contact between grape skin and juice in the production of red wine (Ratola et al., 2004). This study presented that Merlot grape extract had the highest resveratrol content, while Red Globe variety was the lowest, showing a 14fold difference.

DPPH stable free radical scavenging activity

DPPH assay is an excellent tool for monitoring of chemical reactions involving radicals. DPPH is a stable free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (Sun et al., 2011). DPPH is also a well-known free radical, and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Xu et al. 2010). As a rapid and simple measure of antioxidant activity, the DPPH radical scavenging capacity has been widely used. The DPPH assay was based on the reduction of the stable radical DPPH to yellow colored diphenyl picrylhydrazine in the presence of a hydrogen (Longanayaki and Manian, donor 2010). The concentration of the extract necessary to decrease the initial concentration of DPPH by clearance rate under the

experimental condition was calculated and the data presented in Table 2 and the values are significantly different. The scavenging effect of extracts on the DPPH radical decreased in the order of Cabernet Sauvignon > Milk grape > Muscat > Merlot > Cabenet Franc > Vitis labruscana Kyoho > Red Globe > Cabernet Gernischt. The results showed that, although all the samples have noticeable effect on DPPH radical, Cabernet Gernischt was reported to have sustainable hydrogen donating and radical scavenging ability. It has been reported that the antioxidant activity of many compounds of botanical origin is proportional to their phenolics contents, suggesting a causative relationship between total phenolic content and antioxidant activity. Interestingly, Cabernet Gernischt, which exhibited the highest content of total phenolic, total flavonoid and total anthocyanin, registered the highest DPPH radical scavenging potential.

ABTS⁺⁺ scavenging activity

ABTS is frequently used to measure the antioxidant capacities of foods. This ABTS method determines the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. The ABTS assay is applicable to both lipophillic and hydrophilic compounds (Sasidharan and Menon, 2011). The antioxidant capacities of extracts from different samples using the ABTS assay are shown in Table 2. In present study, the extract of all the samples showed notable ABTS⁺ cation radical scavenging activity. Most fruits tested with high antioxidant capacity in the DPPH model, also showed a high antioxidant capacity in ABTS model. A significant correlation also exists between the total antioxidant

Cultivar	DPPH (%)	ABTS (%)	FRAP [mmol Fe(II)/g extract]
Cabernet Sauvignon	92.77 ± 0.52 ^a	95.29 ± 2.18 ^a	1875.1 ± 50.03 ^a
Cabenet Franc	89.30 ± 6.63^{abc}	85.07 ± 3.68^{a}	1040.2 ± 67.94 ^d
Merlot	89.91 ± 2.41 ^{abc}	92.94 ± 2.11 ^{ab}	1214.8 ± 74.95 ^c
Cabernet Gernischt	85.59 ± 2.05 ^c	88.69 ± 1.65 ^{ab}	1542.26 ± 85.18 ^b
Muscat	90.83 ± 1.46 ^{ab}	85.43 ± 4.08^{b}	759.48 ± 7.62^{e}
Red Globe	85.75 ± 6.94 ^{bc}	77.68 ± 4.27 ^b	529.34 ± 19.89 ^e
Vitis labruscana Kyoho	88.13 ± 1.99 ^{abc}	88.57 ± 1.16 ^{cd}	811.36 ± 8.80^{f}
Milk grape	92.28 ± 3.17 ^{abc}	63.27 ± 8.48 ^{cd}	595.26 ± 13.37 ^f

Table 2. DPPH, ABTS⁺ and FRAP scavenging ability of 8 grape varieties.

^{a to f}Bar with no letters in common are significantly different (p < 0.05) in the same column.

capacity and total phenolics and between total antioxidant capacity and total flavonoids. According to Bao et al. (2002), the highest ABTS scavenging activity of bayberry was attributed to the presence of higher levels anthocyanins, flavonoids, and total phenolic compounds. Fruit antioxidants, which include ascorbic acid, tocopherol, and phenolics vary greatly in their contents and profile among various fruits.

FRAP assay

Ferric reducing antioxidant power is an antioxidant capacity assays. The FRAP assay is often used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols. The FRAP assay may offer putative index of antioxidant activity and measure the ability of antioxidants to reduce the ferric 2, 4, 6-tripyridyl-S-triazine complex [Fe(III)- $(TPTZ)_2]^{2+}$ to intensely blue colored ferrous complex [Fe(II)-(TPTZ)2]²⁺ in acidic medium. The antioxidant capacities of extracts from different samples using the FRAP assay are shown in Table 2. The fruit extracts in general exhibited higher antioxidant capacities and the values ranged from 529.34 to 1875.1 mmol Fe(II)/g. The antioxidant capacity of extracts on the FRAP assay decreased in the order of Cabernet Sauvignon > Cabernet Gernischt > Merlot > Cabenet Franc > Vitis labruscana Kyoho > Muscat > Milk grape > Red Globe. Further, FRAP exhibited a significant correlations with ABTS radical scavenging activities. Recently (Oktay et al., 2003), a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. It has been reported that the FRAP of Kei apple fruit juice correlated well with the polyphenol concentrations and the authors suggested that higher antioxidant activity of fruit juice might be due to the presence of phenolics.

The present results describe not only the total phenolic, total flavonoid, total anthocyanins and resveratrol contents of 8 grape varieties (four wine grapes and four table grapes) but also their antioxidant activities. This work has shown that the phenolic present in grapes has

potent antioxidant, and that the antioxidant activity in grapes is positively correlated with total phenolic, total flavonoids, total anthocyanins and resveratrol content. Our results have also found that significant differences in phenolic content can exist among grape varieties. The wine grapes have higher total phenolic, total flavonoid and total anthocyanin content. Cabernet Sauvignon, Cabernet Gernischt and Merlot possessed higher antioxidant capacity. On the contrary, the table grapes have less antioxidant capacity. The oxidative stress arising from an imbalance in the human antioxidant status contributes to the pathology of chronic diseases (Ames et al., 1993). In recent years, many studies have demonstrated that free radicals are the leading cause of degenerative diseases such as several forms of cancer, cardiovascular disease, and neurological diseases. Phenolics have attracted increasing attention for their antioxidant activities. These antioxidants work as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, and enzyme inhibitors. Grapes provide phenolic antioxidants, which contribute to their potential health benefits. The beneficial health-related effects of phenolics in grapes are of importance to consumers, breeders and the grape industry. In the future research work, information on the bioavailability and metabolism of phenolics in humans, which is still scarce, should be considered.

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REFERENCES

Ames BN, Shigenaga MK, Hagen TM (1993). Oxidants, antioxidants

and the degenerative diseases of aging. P. Natl. Acad. Sci. U.S.A., 90: 7915-7922.

- Bao J, Cai Y, Sun M, Wang G, Corke H (2005). Anthocyanins, flavonols, and free radical scavenging activity of chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. J. Agric. Food Chem., 53: 2327-2332.
- Breksa AP, Takeoka GR, Hidalgo MB, Vilches A, Vasse J (2010). Antioxidant activity and phenolic content of 16 raisin grape (*Vitis vinifera* L.) cultivars and selections. Food Chem., 121: 740-745.
- Cao G., Sofic E, Prior RL (1997). Antioxidant capacity and prooxidant behavior of flavonoids: Structure-activity relationships. Free Radical Bio. Med., 22: 749-760.
- Cook NC, Samman S (1996). Flavonoids: chemistry, metabolism, cardioprotective effects and dietary sources. J. Nutr. Biochem., 7: 66-76.
- Herraiz T, Galisteo J (2004). Endogenous and dietary indoles: a class of antioxidants and radical scavengers in the ABTS assay. Free Radical Res., 38: 323-331.
- Hou DX (2003). Potential mechanisms of cancer chemoprevention by anthocyanins. Curr. Mol. Med., 3: 149-159.
- Jia Z, Tang M, Wu J (1999). The determination of flavonoid contents in mulberry and their scavenging effetcs on superoxide racicals. Food chem., 64: 555-559.
- Katalinic V, Milos M, Kulisic T, Jukic M (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem., 94: 550-557.
- Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003). Review:Analysis and biological activities of anthocyanins. Phytochem., 64: 923-933.
- Longanayaki N, Manian S (2010). In vitro antioxidant properties of indigenous underutilized fruits. Food Sci. Biotechnol., 19: 724-734.
- Mulero J, Pardo F, Zafrilla P (2010). Antioxidant activity and phenolic composition of organic and conventional grapes and wines. J. Food Compos. Anal., 23: 569-574.

- Mullins MG, Bouquet A, Williams L (1992). Biology of the grapevines. Cambridge University Press. New York, USA.
- Oktay M, Čulcin I, Kufrevioglu OI (2003). Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensm.-Wiss. Technol., 36: 263-271.
- Ratola N, Faria JL, Alves A (2004). Analysis and Quantification of trans-Resveratrol in Wines from Alentejo Region (Portugal). Food Technol. Biotechnol., 42: 125–130.
- Sasidharan I, Menon AN (2011). Effects of temperature and solvent on antioxidant properties of curry leaf (*Murraya koenigii* L.). J. Food Sci. Technol., 48: 366–370.
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299:152-178.
- Sun Q, Shen HX, Luo YK (2011). Antioxidant activity of hydrolysates and peptide fractions derived from porcine hemoglobin. J. Food Sci. Technol., 48: 53–60.
- Valavanidis A, Vlachogianni T, Psomas A, Zovoili A, Siatis V (2009). Polyphenol profile and antioxidant activity of five apple cultivars grown under organic and conventional agricultural practices. Int. J. Food Sci. Tech., 44: 1167-1175.
- Wang H, Cao G, Prior RL (1997). Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem., 45: 304-309.
- Xu ML, Wang L, Hu JH, Lee SK, Wang MH (2010). Antioxidant activities and related ployphenolic constituents of the methanol extract fractions from *Broussonetia papyrifera* stem bark and wood. Food Sci. Biotechnol., 19: s677-682.
- Yang J, Martinson TE, Liu RH (2009). Phytochemical profiles and antioxidant activities of wine grapes. Food Chem., 116: 332-339.
- Yang J, Meyers KJ, HeideJ, Liu RH (2004). Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. J. Agric. Food Chem., 52: 6787-6793.