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Phenolic Content and Antioxidant Activity in Kansas-grown *Vitis vinifera* and Associated Wines

Kaley Kinnamon

ABSTRACT

Phenolic content and antioxidant activity was examined in three Kansas-grown grape varieties (Chambourcin, Lemberger, and Cabernet Franc) and the wines made out of them from the same crop. Antioxidant activity was measured using a DPPH free radical assay and phenolic content was determined using Folin-Ciocalteu reagent and a gallic acid calibration curve. The minimum phenolic content and maximum amount of residual DPPH were found in the Cabernet Franc wine, at 149±2 mg GAE/100mL and 55±1% respectively. Total phenolic content ranged from 149±2 mg gallic acid equivalents (GAE)/100mL in Cabernet Franc wine to 212±4 mg GAE/100g in Cabernet Franc grapes. Antioxidant activity was expressed as % residual DPPH and ranged from 43±1% in Lemberger grapes to 55±1% in Cabernet Franc wine. There was a strong negative correlation between % residual DPPH and phenolic content, with an R^2 of 0.93.

Keywords: *Antioxidants, Folin-Ciocalteu, Grapes, Phenolic content, Radical scavenging, Wine*

INTRODUCTION

Antioxidants have been shown to have various health benefits because of their ability to quench the free radical production that occurs in the early stages of cancer and aging (Ashok, 1999). In Eastern medicine herbs that are considered antioxidants have long been considered anti-diabetic, neuroprotective, anti-hyperglycemic, antibacterial, antihypertensive, and anti -hyperlipidemic (Cai et al. 2014).

Red wines and grapes have been found to have antioxidant properties, primarily attributable to the large number of phenylpropanoids that they contain (Anesi et al 2015). The cardio-protective and overall health benefits of red wine have been attributed to the presence of polyphenols like resveratrol, catechin, epicatechin and proanthocyanidins (Das et al. 1999) in the grapes used to make the wines. Resveratrol is found in the skin of the grapes, while the other polyphenols are located in the pulp and seeds (Das et al. 1999). Phenolic content and antioxidant activity have both been shown to vary in individual wines when they are at different stages of fermentation (Chirita, 2011).

Wine is categorized based on distinct geographical regions where the grapes are grown, as reflected in the *terroir* concept. Though the Napa region of California is the most well-known wine region in the United States, Midwest-grown grapes also have a prominent place in wine history. Native Midwest rootstock from Cabernet Franc and Chambourcin were used as a graft when, in the late 1800s, a phylloxera infestation nearly decimated the French wine industry. The bacterial resistance of the Midwestern rootsock prevented further infection and saved the French crops from further damage (Pinney 1989).

The purpose of this study is to survey antioxidant activity and phenolic content in grapes versus their fermented wine product, all from the same winery and produced in the same year (2015) in Kansas. This investigation will elucidate how wine processing affects antioxidant activity and phenolic content of specific Kansas-grown grapes. It will also provide a measure of the relationship between antioxidant activity and phenolic content and whether that relationship changes once grapes are turned into wine.

MATERIALS AND METHODS

Chambourcin, Cabernet Franc, and Lemberger grapes and wines were collected from the Grace Hill Winery in Whitewater, Kansas during peak harvest. Chambourcin grapes and wine were collected on August 29, 2015 and the Cabernet Franc and Lemberger grapes and wines were collected on September 11th, 2015. All of the fresh, hydrated grapes were stored in a refrigerator until tests were run. All extractions of grape phenolics were done within 2 weeks of collection.

Ethyl acetate, sodium carbonate, acetone, hydrochloric acid, and sodium hydroxide were provided by McPherson College. Folin-Ciocalteu reagent, gallic acid, and DPPH were purchased from Sigma-Aldrich, Inc. All were stored as designated by MSDS.

Extraction of phenolic compounds

Total phenolics were extracted from grape samples in a two-part process outlined by Sun in 2002. 100g of each type of grape was homogenized with 200 mL chilled acetone in a Waring blender for 10 minutes. Solution was filtered through no. 2 Whatman paper in a Buchner funnel under vacuum. Solution was put in an oven at 45 ^{0}C until about 90% of acetone was

evaporated. Solution was then stored in a freezer until assays were run.

Extraction of bound phenolic compounds

Residue from the vacuum filtration step was hydrolyzed with 20 mL of 1M NaOH at room temperature. The mixture was neutralized with 1M HCl using a pH meter, and then extracted three times using 20 mL of ethyl acetate each time in a separatory funnel. The ethyl acetate layer was evaporated at 45 ^{0}C in an oven. The bound phenolic solution was mixed with soluble phenolic solution and stored in a freezer.

Determination of total phenolic content in grapes

The total phenols in the samples were determined by a modified Folin-Ciocalteu method used in the quantification of phenols in wines (Fotakis, 2012). Reduction of the Folin-Ciocalteu reagent by phenols in the sample results in production of molybdenum-tungsten blue that is measured by a Spectronic Genesys 2 spectrophotometer (Dewanto, 2002). The grape extracts were diluted 1:5 with deionized water. 125 microliters of diluted extract was mixed with 0.5 mL deionized water and 125 microliters F-C reagent and allowed to react for 6 minutes. 1.25 mL of 7% sodium carbonate was added to each solution to raise pH and then the final volume was brought to 3mL with deionized water. Solutions were covered and allowed to sit. After 90 minutes, measurements of absorbance at 765 nm were taken using a Spectronic Genesys 2 spectrophotometer. The standard curve was prepared using known concentrations of gallic acid (0.01-0.05 mg/mL) and results were expressed as mg gallic acid equivalents (GAE) per 100 grams of fresh weight grapes.

Determination of total phenolic content in wines

The modified Folin-Ciocalteu method used above was also used for wines, where wine samples were measured against a gallic acid calibration curve (Fotakis, 2012). 100 microliters of wine, 20 microliters of F-C reagents, and 50 microliters of 7% sodium carbonate were diluted to 500 microliters with deionized water. The mixture was then incubated at 45 degrees Celsius for 15 minutes. Absorbance of each sample was read at 765 nm using a Spectronic Genesys 2 spectrophotometer.

DPPH assays

4.0 mg DPPH was dissolved in 100 mL ethanol and mixed in the dark for 30 minutes. 0.10 mL of wine was mixed with 0.30 mL 90/10% ethanol/methanol solution. 30 microliters of wine solution was mixed with 3.0 mL of DPPH solution and incubated at room temperature for 50 minutes. Absorbance was then measured at 515 nm for the blank (wine+ethanol), control (ethanol+DPPH), and sample. Procedure was repeated with the same diluted phenolic grape extracts used in the phenolic content assay.

RESULTS

The amount of total phenolic content was determined with Folin-Ciocalteu's reagent. Gallic acid was used as the standard and the total phenols were expressed as mg GAE/100g fresh weight of grapes and mg/mL GAE for wines. The equation $y=3.692x$ with an $R^2=.96596$ was found for wines and $y=2.894x$ with $R^2=.99429$ for grapes where y is absorbance and x is total phenolic content. Figures 1 and 2 show the gallic acid calibration curves for grapes and wines.

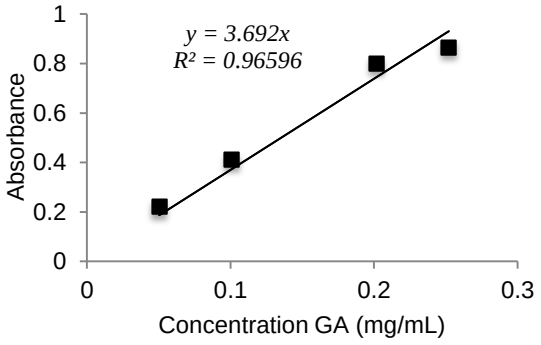

Figure 1. Gallic acid calibration curve for wines

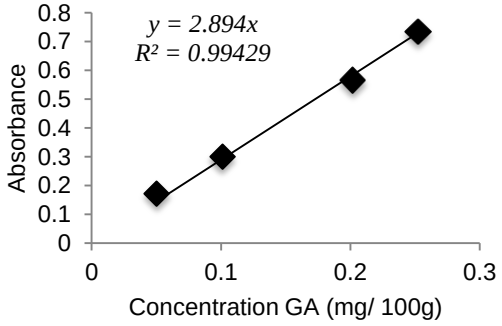

Figure 2. Gallic acid calibration curve for grapes

Table 1 shows the total phenolic content of wine and grape samples in terms of gallic acid equivalents. Total phenolic content ran from 162.9±0.7 to 212±5 mg/100 g extract in the grapes and from 149±2 to 173.0±0.3mg/100mL in the wines. The maximum phenolic content was found in Cabernet Franc grapes and the minimum amount was found in the Cabernet Franc wine.

A DPPH scavenging assay was used as a metric to measure antioxidant activity in grapes and wines. When the purple DPPH free radical is in the presence of antioxidants, the color is diminished due to a donated electron. The loss of color was measured with a Spectronic Genesys 2 spectrophotometer at 515 nm. Maximum residual

Table 1. Total phenolic content (TPC) in grape and wine species. Values are means ± SD (n=3) and in units of mg/100 g for grapes and mg/100mL for wines

Wine	TPC
Chambourcin	173.0±0.3
Lemberger	160±2
Cabernet Franc	149±2
Grape Extract	
Chambourcin	162.9±0.7
Lemberger	196.3±0.6
Cabernet Franc	212±5

DPPH was found in Cabernet Franc wine, at 55±1%. Minimum residual DPPH was 43±1% in Lemberger grapes. The higher the residual DPPH, the lower the quenching power of the sample.

Table 2. Total antioxidant activity in grape and wine species. Values are means ± SD (n=3) and are expressed as % residual DPPH.

Wine	% Residual DPPH
Chambourcin	49.4±0.7
Lemberger	51.6±0.9
Cabernet Franc	55±1
Grape Extract	
Chambourcin	52±1
Lemberger	43±1
Cabernet Franc	43.9±0.8

The results, shown in Figure 3, demonstrate a negative association between % residual DPPH and phenolic content (R^2=.931).

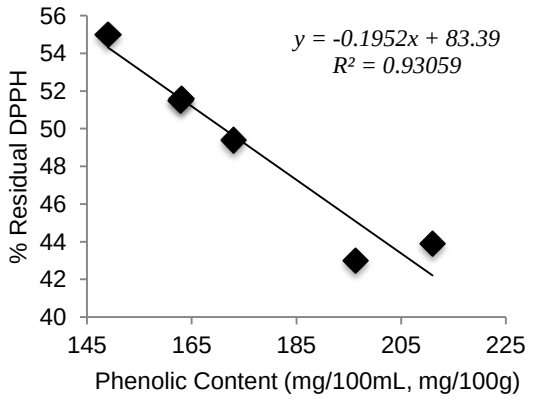

Figure 3. % residual DPPH vs. phenolic content

DISCUSSION

Consumption of red wine has been linked to neuroprotection (Virgili 2000) and cardioprotection (Das 1999) due to the presence of resveratrol and other phytochemicals. Grapes also contain several phytochemicals, including flavanols, phenolic acids, anthocyanins, and stilbenes (a category which includes resveratrol). The amounts of phenolic compounds and antioxidant activity can vary between different species of grapes. They also differ amongst red wine varieties due to different methods of preparation and added contents (Chirita 2011). In this experiment, the relationship between total antioxidant activity and total phenolic content (TPC) was measured in three different wines (Cabernet Franc, Lemberger, and Chambourcin) and their constituent grapes. TPC and antioxidant activity were found to have a positive correlation in this study, shown by % residual DPPH and phenolic content having a coefficient of determination of 0.93. This suggests that total phenolic content is a good predictor of cellular antioxidant activity in grapes and wines. Total phenolic content ranged from 149±2 mg GAE/100mL (Cabernet Franc wine) to 212±4 mg GAE/100g (Cabernet Franc grapes). Antioxidant activity- expressed here as % residual DPPH- ranged from 43±1% in Lemberger grapes to 55±1% in Cabernet Franc wine. In a study by Liang (2014), 24 different grape cultivars were measured, with a range from 95.3 to 686.5 mg GAE/100g grape mass. The difference in phenolic content amongst grapes could be attributed to suitability of location to grape-growing, as Kansas soil is not ideal grape-growing terrain. Wine and grape differences can most logically be attributed to changes made during processing and addition of external materials. The differences in both phenolic content and antioxidant activity between grapes and wines were statistically insignificant, when comparing the means of the two ($p>0.05$). It is known that grapes have high levels of phenolic compounds when compared to other fruits (Sun 2002). Using a TOSC antioxidant assay, Sun et al. determined that cranberries had the highest level of antioxidants among fruits tested, with a value of 177.0±4.3 µmol of vitamin C equiv/g fruit. Cranberries were followed by apples and then red grapes. Red grapes had a value of 64.7±1.6 µmol/g. *Terroir*, environmental conditions, fermentation and processing all affect the TPC of grapes. The information in this study is important for Kansas winegrowers interested in phenolic content, and for those interested in tailoring their processing to get maximum phenolic expression in the wines produced from their grapes.

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