ASSESSING THE TOTAL PHENOLICS OF ROMANIAN WINE VARIETIES

Stoica R.[1,2], Ion R.M.[1,2],. Bunghez I.R [1,2], Senin R.[2], Bajenaru I. G. [2]

[1]Valahia University of Targoviste
[2]National Research and Development Institute for Chemistry and Petrochemistry, ICECHIM Bucharest
E-mail: irusandica@yahoo.com

Abstract. Phenolic compound are ubiquitous constituents of higher plants found in the wide range of commonly consumed plants foods such as fruits, vegetables, cereals and legumes, and in beverages of plant origins, such as wine, tea and coffee (Stoica R., [1]). Phenolics are a large and complex group of compounds of a particular importance to the characteristics and quality of red wine. They are also significant in white wines but occur in much lower concentration. These compounds can affect the appearance, taste, mouth-feel, fragrance, and antimicrobial properties of wine. (Jackson R. J., [2]). Phenolics in grapes and wine include many different substances: phenolic acids, flavonoids, proanthocyanidins and polymeric condensed tannins (Waterhouse A.L., [3]). Measuring these different analytes and reporting meaningful value in a single number it is an analytical challenge.
In this paper is described the total phenolics assessment using Folin-Ciocalteau method widely applied for the analysis of the total phenolics in wine. A global assessment of the total phenolic content of some Romanian wines was achieved.

Keywords: total phenolics, wine, Folin-Ciocalteau method

1. INTRODUCTION

The analysis of the phenolic compounds in wines it is of considerable commercial importance, since they are known to play the major role in defying the sensorial characteristics of wines, such as astringency, flavor and color, as well as in the browning process, causing product deterioration. (*Samanidou, V.F. [4]).*

In white wine, the most important phenolic compounds are the hydroxycinnamic acids and of minor quantities, the flavan-3-ol monomers. These compounds are important with regard to the visual quality of white wine. In red wine, tannins and anthocyanins are the most important phenolic classes. Tannins contribute to the mouthfeel of wines but they also form pigmented polymers in association with the anthocyanins to provide the stable pigments required to give red wine its longterm color stability. (*Kennedy, J.A. [5]).*

These compounds can be classified in a number of ways. In figure 1, they are classified according to the number of phenol subunits. A monomeric subclass consists of the phenolic acids. Although these acids demonstrate comparable antioxidant activity to their polymeric counterparts (i.e. flavonoids), less is known concerning their health or antioxidant benefits. (Robbins, R.J. [6]).

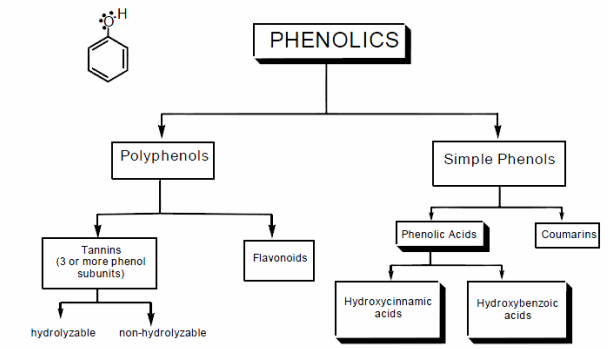

Figure 1. Classification scheme of phenolics according to number of phenol subunits.

2. EXPERIMENTAL

Folin-Ciocalteau (FC) colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. Singleton adapted this method to wine analysis (*Singleton and Rossi, [7])* and has written two major reviews on its use *(Singleton, [8])* and *(Singleton et al., [9])*. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. The FC method has been adopted as the official procedure for total phenolic levels in wine; the Office International de la Vigne et du Vin (OIV), the one international body that certifies specific procedures for wine analysis, accepts the FC method as the standard procedure for total phenolic analysis (OIV, 1990). An earlier variation was the Folin-Denis procedure, but the FC method has displaced it except in

a few historical cases of official procedures that have not been updated (AOAC International, 1995).

Materials

Sample, e.g. white wine or 10% (v/v) red wine in water

Galic Acid calibration standard

Folin-Ciocalteau (FC) reagent

Sodium carbonate solution

100 ml volumetric flask

Spectrophotometer set to 765 nm with 1 cm plastic or glass cuvettes

Wine samples

Wines were purchased from local stores. (1) White wine Muscat Otonel, 2008, Murfatlar vineyard area, ID WW01; (2) Red wine Cabernet Sauvignon, 2009 Murfatlar vineyard area, ID RW01; (3) Red wine Cabernet Sauvignon/Merlot, 2009, Recas vineyard area, ID RW02.

Quantification

0.5 g gallic acid was dissolve in 10 ml ethanol and was diluted to 100 ml with water (5 g/ liter final). From dilution of 1, 2, 5 and 10 ml of stock solution to 100 ml with water was created standards with 50, 100, 250, 500 mg/liter concentration, respectively. These solutions was store up to 2 weeks at 4 ^{0}C.

Sample preparation and measurement

1ml sample, a gallic acid calibration standard, or blank (deionized or distilled water) was placed into 100 ml volumetric flask.

70 ml water was added, followed by 5 ml FC reagent. This was mixed and incubated 1 to 8 min at room temperature.

Then was added 15 ml sodium carbonate solution and water to the 100 ml line, mixed and incubated 2 hr at room temperature.

2 ml was transferred to a 1 cm plastic or glass cuvette and was measured its absorbance at 765 nm in a spectrophotometer.

3. RESULTS AND DISCUSSIONS

The calibration curve was determined from all readings of gallic acid calibration standard absorbance.

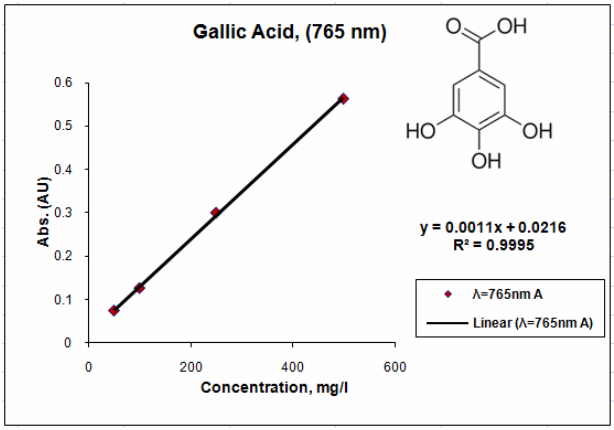

Figure 2. Gallic acid calibration curve.

Under the assay conditions described, a linear relationship between the concentration of gallic acid and the absorbance at 765 nm was obtained. The correlation coefficient for standard curve exceeded 0.99 for gallic acid.

Limit of detection and quantification

Because wines that have total phenol levels lower than 50 mg/l area quite rare, this is not a significant issue for the wine. For other sample types, however, a limit of quantification of □ 0.027AU or 20 mg/l would be expected, based on a sample to sample variance of 0.003AU (Singleton and Rossi, [7]).

Total phenolics content of wine samples analyzed with this method are represented below:

Table 1. Total phenolics content of wine samples

Wine ID	Total phenolics, mg/l GAE
WW01	372
RW01	1890
RW02	2299

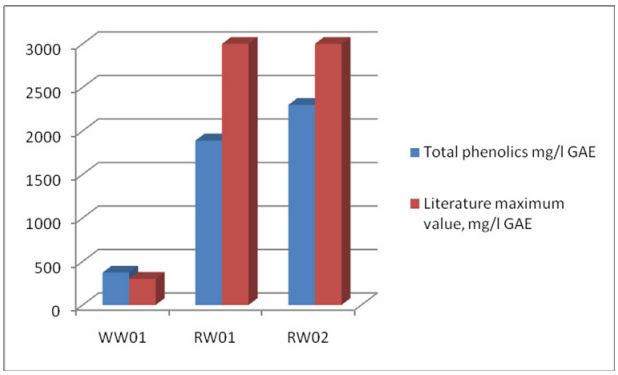

Figure 3. Total phenolics content of wine samples

Because a single substance is used, the result is reported as a response equivalent to the amount or concentration of that substance. As wine is analyzed on a concentration basis, the result is reported in gallic acid equivalents (GAE) using units of mg/liter. For any standard, the results must always be reported on an equivalent basis to avoid the perception that one is measuring the amount of the standard substance.

The level of total phenolics in white wines varies from <100 to 300 mg/liter by the FC method. The levels will be on the low end of the scale if the must was subjected to oxidative treatment and the pressing was very light.
Higher levels will be observed when harder pressing of the solids is utilized or if the wine was aged in new oak barrels.
Red wines have total phenolic levels of 1 to 3 g/liter, with typical average of <1.8 g/liter. [Waterhouse, 3].

Folin-Ciocalteau index for these wines was also determined. The values obtained confirm literature data, being in the range between 10 and 100. (Jackson R. J., [2]).

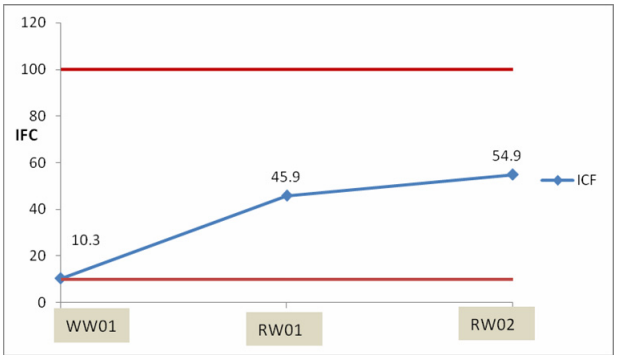

Figure 4. Folin-Ciocalteau value

Differences are due to differing amounts of phenolics in grapes based on variety and growing conditions, with moderate to cooler climates yielding higher levels. Production techniques can have a secondary effect, and longer contact times or higher temperatures will increase the amount of phenolics extracted from the grape solids into the wine.

The results obtained have confirmed a variation in the phenolic content amongst white and red wines, and also amongst wines made from grapes of different geographical origin.

4. ACKNOWLEDGMENTS

This work was partly financed by the POSDRU/CPP107/DMI1.5/S/77497 Program for PhD. Students.

5. REFERENCES

[1]. Stoica R., Ion R.M., Doncea S.M., Trandafir I. An HPLC method for identification and separation of some phenolic acids in the coffee. Bulletin of Petroleum-Gas University of Ploieşti, LXII, No. 3A (2010) pp.143-148.

[2]. Jackson R.J. Wine Science, 2nd Ed. Chemical Constituents of Grapes and Wine (2000), pp.248

[3]. Waterhouse A. L. Determination of Total Phenolics. In D.-O. a. Y.Lee, Current Protocols in Food Analytical Chemistry (2002), I1.1.1-I1.1.8. California, John Wiley & Sons, Inc.

[4]. Samanidou V.F., Antoniou C.V., and Papadoyannis I.N. Gradient RP-HPLC determination of free phenolic acids in wines and wine vinegar samples after SPE, with photodiode array identification. J.Liq. Chrom.& Rel. Technol., (2001), pp.2161-2176.

[5]. Kennedy J.A. Grape and wine phenolics: Observations and recent findings. Cien. Inv. Agr. (2008), 35(2), pp. 77-90.

[6]. R.J. Robbins, Bean. S.R. Development of a quantitative high-performance liquid chromatography-photodiode array detection measurement system for phenolic acids, Journal of Chromatography A, (2004) 1038, pp.97–105.

[7]. Singleton, V.L. and Rossi, J.A.. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. (1965), 16, pp.144-158.

[8]. Singleton V.L. Analytical fractionation of the Phenolic substances of grapes and wine and some practical uses of such analyses. In Chemistry of Winemaking (A.D. Webb, ed.) (1974) pp. 184-211. American Chemical Society, Washington, D.C.

[9]. Singleton, V.L., Orthofer, R., and Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol. (1999), 299, pp.152-178.