

ANALYSIS OF POLYPHENOLS IN WINE AND JUICES SAMPLES BY UV-VIS SPECTROPHOTOMETRY

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Abstract

Polyphenols are secondary metabolites of plants being involved in the protection against the solar ultraviolet radiation or pathogens aggression and are considered to possess beneficial effects on human health. The paper aims to determine the total concentration of polyphenolic compounds and anthocyanins in various samples such as wine, nectar, coffee and Noni juice, by UV-VIS spectrometry. The analysis of these compounds involves more aspects: determination of polyphenolic substances by the Folin-Ciocalteu method, determination of anthocyanins by the sodium bisulfite method for wine samples.

Introduction

Polyphenols are natural compounds containing phenol groups playing different roles in the vegetals and animal organisms such as antiviral, antiallergic, antiinflammatory, antitumor and antioxidant agents [1]. Polyphenols are found in fruits, vegetables, cereals and beverages. Fresh fruits, such as grapes, apples, pears, cherries and berries, contain up to 200-300 mg of polyphenols in 100 grams of fresh product [1-3].

Anthocyanins are water-soluble pigments responsible for blue, purple and red colors in many fruits such as blackberries, grapes, cherries, pomegranates, plums, apples, and some citrus and tropical fruit [4-5]. The Folin-Ciocalteu Reagent is also known as "Folin's phenolic reagent" or "gallic acid equivalent" (GEA). It is a mixture of phosphomolybdate and phosphotungstic used in the colorimetric and spectral analysis of phenolic and polyphenolic antioxidants. The GEA reagent has the advantage of the ability to reduce phenolic compounds in samples of different concentrations of polyphenols [6-11]. The method for determining anthocyanins consists in the property of anthocyanins and compounds containing flavil groups to be discolored by sulfur dioxide. The determination of the potassium permanganate index consisted in the cold titration of polyphenolic substances and other oxidizable substances contained in wine against a tartaric and alcoholic solution in the presence of carmine indigo as an indicator of oxidoreduction with potassium permanganate [12-18].

Experimental

In the experiment, table red wine, blackcurrant nectar from the market made with blackcurrant juice concentrate (minimum 50%), coffee, Noni juice (a Tahitian drink appreciated for its antioxidant qualities) were used in the experiment to analyse their polyphenol concentrations.

The blank solution and the standard solutions were prepared. The blank solution was prepared using 1 mL of distilled water, 20 mL of anhydrous 20% Na₂CO₃, 5 mL of Folin-Ciocalteu reagent, and finally the flask is filled to the volume of 100 mL with distilled water. The 5 standard solution was prepared using 1 mL of gallic acid of various known concentrations (50, 100, 150, 250, 500 mg / L), 20 mL 20% anhydrous NaCO₃, 5 mL Folin-Ciocalteu reagent in a

flask of 100 mL. A complex of blue color is formed and their absorbance is measured after 30 minutes. The standards were analysed measuring their absorbance in 1 cm cuvettes at 760 nm. UV-VIZ analysis was performed with a Perkin Elmer, Lambda 25 spectrophotometer. The samples were diluted in an appropriate ratio of 1:20 or 1:10 volume and then they were treated as the standard solution of galic acid with Na_2CO_3 20% solution and Folin-Ciocalteu reagent and by measuring their absorbance at 760 nm after 30 minutes. Samples of red wine, blackcurrant nectar, coffee and Noni juice were thus analyzed to find their polyphenol content. The anthocyanins content analysis in wine based on their decoloration with sodium bisulfite solution was performed for some wine samples. The method of determining the anthocyanins was to add 1 mL of ethanol with 1% HCl, 1 mL of wine and 20 mL of 2% hydrochloric acid to a flask. After homogenizing the mixture, 10 mL of the prepared mixture and 4 mL of distilled water are pipetted into the tube A, and 10 mL of the prepared mixture and 4 mL of 15% sodium bisulfite are pipetted into tube B. After homogenization and 15 minutes rest, sample extinctions at 520 nm were determined in a 10 mm cuvette. The results obtained were recorded with e1 (extinction of the sample with distilled water), respectively with e2 (extinction of the sample with sodium bisulfite). The difference between these values was multiplied by 875, which is a set point from the calibration right obtained with purified anthocyanins from wine. For this experiment, table wines, semi-sweet, semi-sweet and sweet wines were used in 2016.

Results and discussion

After calibration, the calibration curve of Figure 1 was plotted. The determination coefficient R^2 of 0.9919 suggests a very good correlation of the data.



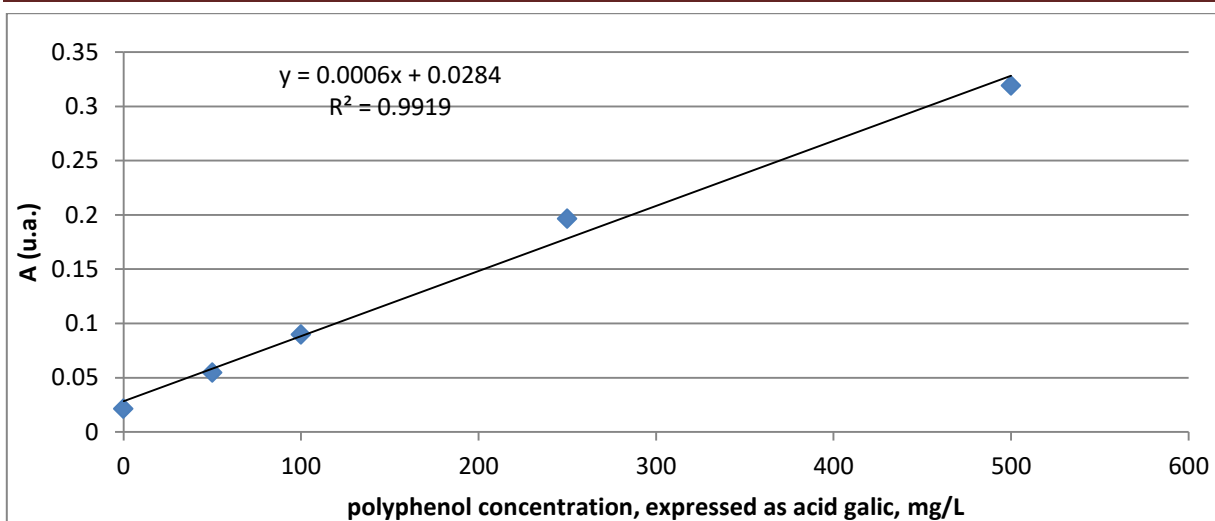


Figure 1. The etalons of galic acid for polyphenol analysis based on Folin-Ciocalteu reagent

The samples thus prepared were analyzed spectrophotometrically at the wavelength of 760 nm, yielding the results presented in Table 1.

Table 1. Polyphenol content of the analysed samples

Sample	Polyphenol content, mg/L
Red wine	419±23.63 ^a
Black currant juice	1368.33±64.85
Coffee (as drink)	4513.33±76.70
Noni juice	2747±51.28

^a standard deviation

It can be seen from the table that both wine and nectar from black currants, but especially coffee and Noni juice, contain high concentration of antioxidant substances of the polyphenol nature. The very high concentration of polyphenols in coffee samples is explained by the fact that it was prepared a few hours before the experiment and was prepared in a concentrated manner.

The concentration of anthocyanins in wine samples by the spectrophotometric method is shown in Table 2. Table wine has the same provenance as the wine used in the first experiment (the total amount of polyphenols determined was 419 mg / L).

Table 2. The anthocyanin concentration in different wine assortments

Type of sample	Anthocyanins concentration (mg/L)
Table wine	9.58±0.88
Demidry wine	82.75±5.68
Demisweet wine	145±4.41
Sweet wine	167.82±4.78

The weight of anthocyanins in total polyphenols for the first type of wine is 2.28%. The highest concentration of anthocyanins was found for the sweet wine sample followed by the demiwseet wine while table wine sample was poorer in polyphenols.

Conclusion

Polyphenolic compounds are particularly important in the living world, found in all plants, being responsible for the physiological processes inside the organisms that contain them. The structure of anthocyanins plays a determining role in the color formation of fruits, producing shades from red-orange (pelargonidine) to blue-violet (delphinidin). Hydroxylation induces a turning effect of the visible maximum to higher wavelengths, respectively turning to blue. On the other hand, methylation of hydroxyl groups has a counter-effect, producing red-intense colors. Due to their particular spectral properties it is possible to determine their presence, concentration and evolution in the substances that contain them by means of the UV-VIS molecular absorption method. The studies undertaken in the paper provide information and give us the certainty that a greater variety of determinations can be made with this method.

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