EVALUATION OF PHENOLIC COMPOUNDS BY AN ECOLOGICAL SPECTROMETRIC METHODS

EVALUAREA COMPUŞILOR FENOLICI PRIN METODE SPECTROMETRICE ECOLOGICE

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Abstract. *The study aimed to analyse phenolic compounds from wine samples using "ecological" spectrometric methods (low consumption of chemical reagents) comparing a traditional UV-VIS spectrophotometer to a microplate reader spectrometer. Different experimental wine samples were obtained using active charcoal, gelatine and anionic resins. The used wines were produced from Feteasca neagră grapes harvested at full maturity from Şuletea region, Vaslui County, with approx. 290 g/L concentration in fermenting substances. A total of 43 samples were analysed. The microplate reader was shown to be an extremely efficient and economic tool for analysing wines as the linearity of the used methods was shown to be comparative between devices. The time required for analysis is considerably reduced in this case and the versatility of the method allows better statistical evaluation of parameters within the limit of 10% of the relevant significance.*
Key words: phenolic compounds; spectrophotometry; plate reader

Rezumat. *Lucrarea îşi propune analiza compuşilor fenolici din probe de vin prin metode spectrometrice de tip „ecologic" (consum redus de reactivi chimici), utilizând un spectrofotometru tradiţional UV-VIS în comparaţie cu un Spectrofotometru cu micro-plăci. În acest sens, au fost utilizate 43 probe reprezentând vin din soiul Fetească neagră, variante tratate cu cărbune activ, gelatină şi răşini anionice. Strugurii utilizaţi la vinificare au fost recoltaţi la maturitate deplină din regiunea Şuletea, jud. Vaslui având o concentraţie în substanţe reducătoare de 290 g/L. Spectrofotometru cu micro-plăci s-a dovedit a fi un instrument extrem de eficient şi rentabil pentru a analiza vinurile roşii deoarece s-a demonstrat liniariatatea comparativă a metodelor investigate. Se reduce astfel considerabil timpul necesar desfăşurării analizelor comparative, iar versatilitatea metodei permite o mai bună evaluare statistică a unor factori direcţi în limita a 10% din semnificaţia relevantă.*
Cuvinte cheie: compuşi fenolici, spectrofotometrie, spectrofotometru cu microplăci

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INTRODUCTION

Grapes and wine contain a diverse group of phenolic compounds that serve as important oxygen sources and as substrates for browning reactions (Zoecklein *et al.,* 1990). The phenolic concentration in wines depends on many factors, including vineyard environment, wine-making technique and grape variety (Jungmin and Tarara, 2007).

Polyphenolic compounds are responsible for the quality of wines, especially red wines, with a major influence on antioxidant activity, astringency, bitter sensation and colour, their concentration in wine being influenced by variety, technology process and of course, analysis method. Measurement of wine phenolic compounds in red wine is an important part of quality control in beverage industry. Conventional methods are time consuming, include laborious process of reading individual samples in separate cuvettes, recording the results and analysing the values obtained, with large amounts of solvents and reagents, totally un-ecological. In this case, the need to identify a rapid and cost-effective way of performing these tests was stringent. The microplate reader represents an innovative way of normalizing the absorbance analysis in microplate wells (Attard, 2013).

MATERIAL AND METHOD

Chemical parameters' analysis were performed according to International Organisation of Vine and Wine methods of analysis. 43 samples representing wines obtained from Fetească neagră variety were tested. The grapes used for wine-making were harvested at full maturity from Şuletea region, Vaslui county.

In this article, the phenolic compounds of wines were analysed, the values collected with a microplate reader were compared with the values obtained using a traditional spectrophotometer. Different methods for quantification of phenolic compounds were used. Total phenolics and total anthocyanins were determined. The samples were analysed twice, at six months difference and the results were compared.

1. Total phenols were determined by reading the absorbance of the samples at 280 nm. This test is fast and efficient but certain molecules (cinnamic acids, chalcones) have no maximum absorption at 280 nm (Lorrain *et. al.,* 2013).

Another method for total phenolic concentration is the Folin-Ciocalteu test, based on the phenolic compounds with reductive properties. Phenolic compounds are oxidized by Folin Ciocalteu reagent. The blue coloration resulted has a maximum absorption at around 750 nm. Total phenols were expressed as mg gallic acid/L.

2. Tannins. Tannins were determined using the methyl cellulose precipitate tannin assay (MCP). The assay is based on polymer-tannin interactions - the formation of insoluble polymer tannin complexes that precipitates. The assay is based on subtracting the absorbance values recorded at 280 nm and not interfering with the assay. The epicatechin equivalent calibration curves were established on the UV-VIS traditional spectrophotometer that will be used for the tannin assay.

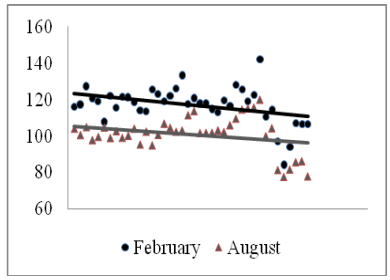
3. Anthocyanins. Anthocyanins content was determined by the pH-differential method, absorbance was read at 520-700 nm. The method is based on the change in

absorbance at 2 different pH values (0.6; 3.6). The total antocyanins content was expressed as Mv-3-gl mg/L.

4. Colour determination. The chromatic parameters of wine samples were calculated according to the CIE, using attributes of specific qualities of visual sensation: clarity, tonality, luminosity, chroma, saturation, hue (OIV-MA-AS2-11). Distilled water was used as a blank sample. Each sample solution was run in triplicate.

RESULTS AND DISCUSSIONS

The method of photometry is different in a microplate reader in comparison to a traditional spectrophotometer. It is necessary to calibrate the path length in the microplate to imitate the traditional method. In horizontal photometry, this is determined by the dimensions of the cuvette (1 cm thickness), the absorbance of a solution being analysed by transmitting light through the sample horizontally, but in vertical photometry, the path length is dependent on the volume in the well and adjustments need to be made (Heredia *et al.,* 2006). Generally, in the microplate method, because wells are too small for adequate blending, most of the incubation has to be performed in a tube and transferred to the microplate. The rest of the protocol is similar.

a) 

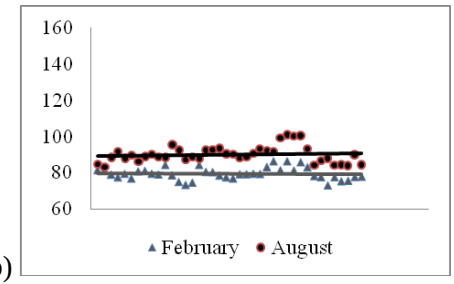
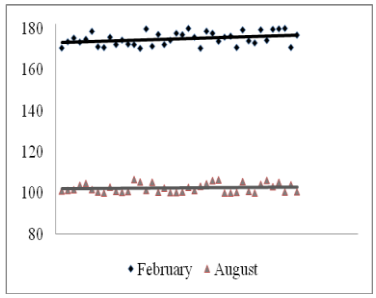
b) 

Fig. 1 Total phenolic compounds (mg/L gallic acid) – microplate reader (a) vs conventional UV-VIS spectrophotometer (b)

a) 

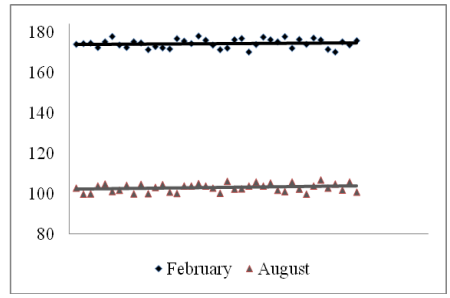
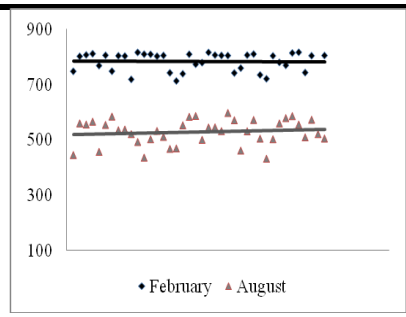
b) 

Fig.2 Folin Ciocalteu index (mg/L gallic acid) – microplate reader (a) vs conventional UV-VIS spectrophotometer (b)

a) 

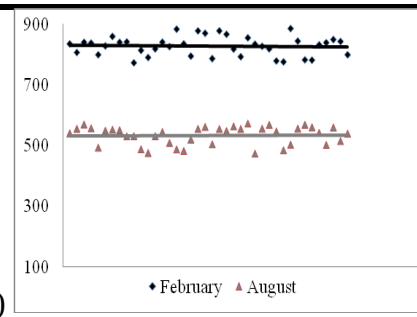
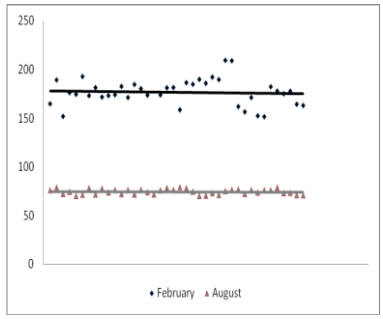
b) 

Fig 3 Tannins content (mg/L epicatechin) - microplate reader (a) vs conventional UV-VIS spectrophotometer (b)

a) 

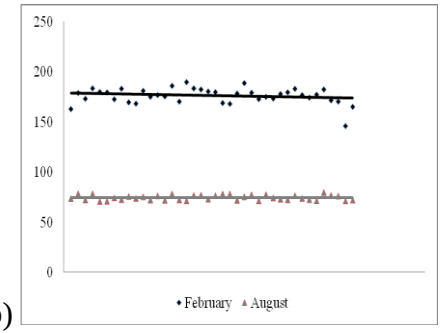
b) 

Fig 4 Anthocyanins content (mg/L Mv-3-gl) - microplate reader (a) vs conventional UV-VIS spectrophotometer (b)

Conventional methods are time consuming and laborious processes of reading individual samples in separate cuvettes, recording the values obtained and analysing the results and needs large amounts of solvents and reagents (Bobo-García, 2014). For example, it takes about 5 minutes to prepare a sample and read the absorbance on a traditional spectrophotometer. The microplate method saves time, reduces the quantity of sample, a higher number of samples can be processed in one experiment and also the repeatability of the results is improved, while also less reagents and solvents are consumed. Another advantage of using microplate reader is the low cost of the system and its energy saving profile (Zhang *et al.,* 2006; Galgani and Bocquene, 1991).

The values obtained with the microplate reader were more precise. The microplate reader allows for a variety of experiments to be measured simultaneously. The software is easy to use, with minimal instructions. Cuvette and microplate methods show different sensitivity and limits of detection (Horswald *et al*., 2011). Microplate method can identify wine components at extremely low concentration. A traditional UV-VIS spectrophotometer is versatile as it can operate at any wavelength between 200 nm and 950 nm. In the case of microplate method, usually the standard has fixed wavelengths, the versatility of this method consisting in the ability to read a large number of samples in a very

short time (Attard, 2013). The majority of data has a linear relationship, the correlation of the results obtained is shown in figures 1, 2, 3 and 4. A positive correlation of the values obtained can be observed. The highest error was recorded for total polyphenols and tannins content. A first reason would be the material of the cuvette. For the spectrophotometer quartz cuvette were used, while for the microplate reader, the cuvettes are made from plastic materials. This may cause differences between the absorbance obtained with the microplate reader and those obtained in the quartz cuvette with the traditional spectrophotometer. In that case, for UV parameters (D280, IFC, Tannins) it is preferable to use the traditional UV spectrophotometer and quartz cuvettes. The microplate method is a faster alternative for the determination of phenolic compounds in various samples (Attard, 2013).

New microplates with used microplates were used in parallel; the results obtained did not show significant differences. Differences in results may also be due to laboratory errors, or samples that containe particles in suspension that influence correct reading.

CONCLUSIONS

1. Spectrophotometry seems to be more accurate and gives the possibility to record a spectrum.

2. The cuvettes material significantly influences the absorbance obtained. For UV parameters (D280, IFC, Tannins) it is preferable to use the traditional UV spectrophotometer and quartz cuvettes.

3. The microplate method is a faster alternative for the determination of phenolic compounds. Microplate method can identify wine components at extremely low concentration. The microplate reader was shown to be an extremely efficient and economic tool for analysing wines because the linearity of the investigated methods was demonstrated to be comparative between devices. The versatility of this method consists in the ability to read a large number of samples in a very short time.

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