INVESTIGATIONS ON THE CORRELATIONS BETWEEN POLYPHENOL CONTENT FROM RED WINES AND THEIR ANTIOXIDANT CAPACITY

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ABSTRACT - *Wine has been identified as a potentially beneficial health-promoting product, due to its effect on coronary heart disease (the so-called "French-paradox"), on the delay of tumor onset and its high antioxidant activity. These benefits have been ascribed to the phenolic compounds, which are abundant in red wine. This research work showed the relationship between antioxidant capacity and the polyphenol total content of Spanish red wines, sampled from Tierra del Arlanza region from Spain. Wines were analysed for the total phenol content by Folin – Ciocâlteu and Mazza methods. Anthocyans and catechols from selected Spanish red wines were quantified. The antioxidant capacity of wines was pointed out by FRAP and DPPH methods. The results of FRAP and DPPH methods were in accordance with an important number of works from this research field.*

Key Words: antioxidant activity, polyphenols, anthocyans, catechols, FRAP and DPPH methods

REZUMAT – Metode de investigaţie a corelaţiilor dintre conţinutul în polifenoli din vinurile roşii şi capacitatea lor antioxidantă. Vinul a fost identificat ca fiind un produs benefic pentru sănătate, datorită efectelor sale asupra afecţiunilor cardiovasculare (aşa-numitul „paradox francez"), întârzierii tumorilor incipiente, având activitate antioxidantă remarcabilă. Aceste beneficii au fost puse pe seama polifenolilor, care se găsesc din abundenţă în vinurile roşii. Lucrarea prezintă relaţia dintre capacitatea antioxidantă şi conţinutul total de polifenoli din vinurile roşii de Spania, colectate din regiunea Tierra del Arlanza, Spania. Pentru analiza conţinutului total de polifenoli s-au folosit metodele Folin-Ciocâlteu şi Mazza. De asemenea, au fost cuantificate catechinele şi antocianii din vinurile roşii de Spania. Capacitatea antioxidantă a fost pusă în evidenţă prin metodele FRAP şi DPPH. Rezultatele obţinute cu cele

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două metode FRAP şi DPPH sunt în concordanţă cu un important număr de lucrări din acest domeniu de cercetare.

Cuvinte cheie: activitate antioxidantă, polifenoli, antociani, catechine, metodele FRAP şi DPPH

INTRODUCTION

According to the "French paradox", a moderate consumption of wine during meals (the Mediterranean diet) justifies the diminution in the mortality index, by coronary diseases. This cardiovascular protection is attributed to polyphenolic compounds present in red wines (Burns et al., 2000).

The polyphenolic compounds protect the biologic systems against free radicals, having the capacity to chelate metals with catalytic trait, such as iron and copper (Yang et al., 2001). *In vitro* research has shown that many natural polyphenols are better antioxidants than E and C vitamins (Leighton, Urquiaga, 2000). Among the great family of polyphenols, flavonoids play an important role. Bioflavonoids are biologically active compounds that, once inside the human body, determine a positive biologic response (Segal, 2006). Bioflavonoids are recommended as supplement in preventing and treating those deficiencies that involve capillary fragility and permeability.

The relationship between the antioxidant activity (or the antioxidant potential) and polyphenolic compounds in wines has been studied by many scientists (Frankel et al., 1995; Cano, Guerrero, 1999; Arnous, Makris, 2002).

From these groups, we have analysed in our laboratory the flavonoids, more precisely the catechols.

Within the same research scope are circumscribed the laboratory tests conducted on a representative sample of 43 red wines from Arlanza region (Spain), for which we have analysed the content in polyphenolic compounds, in catechols, and the antioxidant activity of these wines.

MATERIALS AND METHODS

The wine samples used were commercial wines from the above-mentioned region, of the 2002, 2003 and 2004 vintage. Wines were not previously prepared, just diluted water was applied. The materials used in our experiments were Folin reagent (a mixture of phosphomolibdenic acid and phosphowolframic acid), gallic acid, malvidin 3-glucoside (Extrasynthese, Genay Cedex, France), ethanol, HCl, D- catechols and vanillin.

In order to determine the amount of polyphenolic compounds, we have used the following spectrophotometric methods: Folin-Ciocâlteu, Paronetto and Mazza. The equipment used was a UV-VIS Beckman spectrophotometer DU 650.

1. Determining total polyphenols (TP) by using the Folin-Ciocâlteu method

The method is based on the oxidation reaction between polyphenolic compounds with the Folin-Ciocâlteu reagent. The intensity of the blue colour was proportional to the

content in polyphenolic compounds. The reaction between polyphenolic compounds and the Folin-Ciocâlteu reagent took place in basic pH.

2. Determination of total polyphenols (TP) by using the Mazza method

The Mazza method is used for determining polyphenolic compounds (Mazza et al.,1999). Wine samples were diluted 1:10 with 10% ethanol. As sample, ethanol was then added 95% with 0.1 % HCl and HCl 2%. After homogenization and colour development, the absorbance was read at 280 nm. The gallic acid in 10% ethanol was used as standard solution for the standard curve.

3. Determination of total anthocians

In order to quantify the total anthocians, Paronetto applied the spectrophotometric method (Paronetto, 1977). The method is based on determining anthocyans by using the pH variation of the system. For the calibration curve, we have used malvidin-3-glucozide. The absorbance was read at 525 nm. The read absorbance is interpolated on a calibration curve.

4. Determination of total anthocyans by using the Mazza method

For determining the total anthocyans by using this method, the same reagents as above were used, and the absorbance of samples was read at 520 nm, by using distilled water as blank. To draw up the standard curve, we have used the malvidin-3-glucoside as standard substance.

5. Determination of catechols

The catechols content was estimated by using the Swain and Hills method (Swain, Hills, 1959). The method is based on phenol property of giving condensation reactions with carbonylic groups, in pH acid medium.

For determination, the vanillinic aldehyde (vanillin) was used for calibration, as a carbonyl compound that reacted with "activated" benzene groups of catechols, releasing a red chromophore that is read at 500 nm. For the calibration curve, the D- catechol was regarded as standard substance.

6. Chemical methods of determining the antioxidant activity

For the study of antioxidant activity of the polyphenolic compounds present in the red wines samples, we have used the chemical methods, that is FRAP and DPPH methods.

Generally, the antioxidant activity is given in Trolox milimole (acid 6-hydroxi-2,5,7,8-tetramethylcroman-2-carboxilic), which is a hydrosoluble analogue of vitamin E milimole, ascorbic acid or quercitin (Zaporozhets, 2004). In case of wines, some authors expressed the antioxidant activity in gallic acid equivalents (Minussi et al., 2003).

6.1. FRAP Method (Ferric Reducing /Antioxidant Power)

This method measures the reducing power of the sample with the Fe (III) tripiridyltriazine complex (TPTZ) (Benzie, Strain, 1996). FRAP is based on the reducing capacity of Fe (III) to Fe (II), by the reaction:

$$Fe\ (III)\text{-}TPTZ + 1e^- \rightarrow Fe\ (II)\text{-}TPTZ$$
$$\text{colourless} \qquad\qquad \text{blue}$$
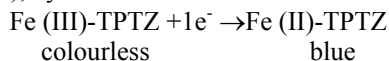
By means of an electron donor (antioxidant), a blue-coloured compound is formed, showing a maximal absorption at 593 nm.

6.2. DPPH Method

The method of sequestrating the DPPH radical, described by Brand-Williams (Brand, 1995), is based on generating free radicals, starting from a methanolic solution of

2, 2 diphenyl-1 picryl-hydrazyl (DPPH), which shows a maximum of absorption at 517 nm. In the presence of an antioxidant agent or a radical, absorption disappears.

The chemical reactions that take place are as follows:

$$DPPH• +AH →DPPH-H +A$$
$$DPPH•+R• → DPPH-R$$
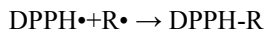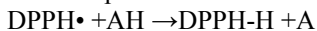
The red wines, which antioxidant activity was studied by using this method, were diluted 1:50 with distilled water. With the obtained values measured, the absorbance difference, ΔA is calculated:

$$\Delta A = A_0 – (A_f – A_{blanc})$$

The calibration curve is given by the equation:

$$\mu g \text{ Trolox} = 8.6166 (A_0 – A_f) -0.095$$

The result is given in μg Trolox/ mL sample.

7. Statistical methods used

The statistical analysis of the experimental data is a fundamental part of research activity and allows simplifying the obtained results, facilitating understanding and further interpreting the obtained results. This was done by using the Software Statgraphics 4.0 for Windows. Statistical pretreatment was done by the Box and Whisker Plot analysis, in order to identify abnormal data, which are far from the average value for each analysed parameter. The statistical program used allows the differentiation between the points possibly abnormal and the ones that are abnormal. Regression was used as a statistical method in processing experimental data.

RESULTS AND DISCUSSION

In order to measure the total polyphenols, we have used the Folin-Ciocâlteu reagent that reacts with reducing substances in the presence of sodium carbonate 75%. The colour intensity was read at 750 nm. For red wines, the operated dilution was of 1:10. The equation of the calibration curve $Y=2.1422·10^{-3}·X +0.0188$; $r^2 =0.9997$ allows us to calculate the quantities of total polyphenols (X) from the absorbance measurements (Y). The results of absorbance measurements were processed by the regression method.

The obtained results of total polyphenols were plot vs. the results of the antioxidant capacity obtained by FRAP and DPPH methods. The correlation between the analysed parameters is shown in *Table 1*.

According to the estimated catechol content, two calibration curves were used, among which, one showing a catechol concentration of 0-500 mg/L, which equation is:

$$Y=2.9353·10^{-3} · X+0.1484,   r^2 =0.9913$$

From this equation, the concentration of catechols, $C_{catechols}$, was calculated:

$$C_{catechols} = (Abs-0.1484)/ 2.9353*10^{-3} \text{ mg/L}$$

In the case of smaller values until 50 mg/L, a calibration curve of equation is used:

$$Y=6.3714·10^{-3} ·X+1.5908·10^{-3}   r^2=0.9991$$

The obtained results of catechols were plot vs. the results of the antioxidant capacity, obtained by FRAP and DPPH methods. The correlation between the analysed parameters is shown in Table 1, too.

Total anthocians are determined by using the calibration curve:

$$Y = 4.668 \cdot 10^{-3} \ X, \ r^2 = 0.9988$$

Table 1

Correlations (r^2) between the studied variables on wine samples

	TP (Folin)	TP (Mazza)	Anthocyans (Paronetto)	Anthocyans (Mazza)	Catechols	Antiox. activ. DPPH•	Antiox. activ. FRAP
TP (Mazza)	0.974	1.000					
Anthocyans (Paronetto)	0.546	0.636	1.000				
Anthocyans (Mazza)	0.594	0.705	0.937	1.000			
Catechols	0.966	0.945	0.481	0.521	1.000		
Antiox. activ. DPPH•	0.919	0.914	0.488	0.566	0.914	1.000	
Antiox. activ. FRAP	0.959	0.965	0.569	0.615	0.956	0.934	1.000

TP – total polyphenols

The results of total anthocians, obtained by both Paronetto and Mazza methods, were compared to the antioxidant capacity of red wines. The correlation coefficient is shown in *Table 1*. Generally, the methods of determining the antioxidant activity are based on the artificial addition of a free radical in the sample, and afterwards the concentration of the latter one is measured according to the reaction with the antioxidant compounds present in the sample, the polyphenolic compounds in our case. The spectrophotometric measurement is proportional to the reducing capacity of the sample.

In the FRAP method, the absorbance of samples is read at 593 nm, by using acetate buffer as blank. The reaction kinetics was studied considering that, after 30 minutes, the finality of the reaction was reached. For each sample four replicas are done. The calculation of the reducing power is done as follows: a calibration curve is obtained, starting from reducing solutions of copper sulphate (II).

The equation of calibration curve is:

$$Y = 0.6445X + 0.0189, \text{ where } r^2 = 0.9942$$

The concentration in copper sulphate is represented on abscissa, and on ordinate - the difference of absorbance $\Delta A = A_{sample} - A_{blank}$. The blank is represented by the TPTZ reagent without the sample.

The calculation formula is:

$$mMCu(II) = (\Delta A - 0.0189)/0.6445$$

In the DPPH method, the absorbance measurements were done at 517 nm, because the used reagent, radical 2,2 diphenyl-picryl-hydrazyl shows a maximum of absorption at 517 nm. The results obtained for all wines were compared by the regression method and correlated with the content of polyphenols, catechols and anthocians from wines (*Table 1*).

The statistical method and, especially, the regression method were extremely useful in appreciating the correlation between the studied parameters and drawing conclusions on the content in total polyphenols, anthocyans, catechols of the studied wines, as well as the antioxidant activity, determined by the two analysed methods. Finding a good regression coefficient (r^2) can provide us with information on the relationship between variables. According to the value of the regression coefficient, conclusions can be drawn on the relationship between variables.

$0.5 \leq r^2 \leq 0.75$ there is medium intensity connection;

$0.75 \leq r^2 \leq 0.95$ there is a good connection;

$0.95 \leq r^2 \leq 1$ there is a functional connection.

Our wine sample was relevant, and the incidental abnormal data were removed from the correlation matrix.

CONCLUSIONS

The Mazza method is an alternative to the Folin-Ciocâlteu method for determining the total polyphenolic compounds ($r^2 = 0.97$).

The Mazza method is an alternative to the Paronetto method for determining the total anthocyans in red wines ($r^2 = 0.94$).

There is a very good connection between the antioxidant activity, determined by using the FRAP method, and the content in total polyphenolic compounds ($r^2 = 0.96$).

There is a very good connection between the antioxidant activity, determined by using the DPPH● method, and the content in total polyphenolic compounds ($r^2 = 0.92$).

Among the studied polyphenols, catechols show the greatest antioxidant activity ($r^2 = 0.91$ for the correlation between the antioxidant activity, determined by the DPPH● method and the catechols content of the analysed red wines, and $r^2 = 0.96$ for the correlation between the antioxidant activity, determined by the FRAP method).

Both the FRAP and the DPPH● method can be used in determining the antioxidant activity of red wines, thus obtaining results that are very close (the statistical correlation of parameters obtained by the two methods is very good $r^2 = 0.93$).

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