COMPARATIVE STUDY OF ANTIRADICAL PROPERTIES OF SOME ROMANIAN WINES FROM THE COMMERCIAL PRODUCTS

STUDIU COMPARATIV AL PROPRIETĂȚILOR ANTIRADICALICE LA UNELE VINURI ROMÂNEȘTI DIN OFERTA COMERCIALĂ

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Abstract. *Assessment of the bioactive properties of productsis important as well as assessing the amount of alcohol from wines. This property aroused the interest of researchers in the last 3 decades because the literature shows awide range of bioactive phenolic compounds and there positive properties. The study presents two methods for evaluating the antiradical potential of wineas a complex product to consumers. By contrasta suite of phenolic substances with beneficial effects of the product are presented. We analyzed four white and one red wine commercially available. Results varywidely but are higherthan some observations from laborator ymicro-samples.*
Key words: *commercialwine, DPPH, chemiluminescence, phenolic compounds*

Rezumat.*Evaluarea proprietăților bioactive ale unor produse este la fel de importantă precum și evaluarea cantității de alcool a vinurilor. Această proprietate a stârnit în ultimele 3 decenii interesul cercetătorilor deoarece datele din literatură prezintă o serie largă de compuși fenolici cu proprietăți bioactive. Studiul de față prezintă două metode de evaluare a efectului antiradicalic pe care vinul ca un complex îl prezintă pentru consumatori. Prin contrast este prezentată suita de substanțe fenolice are formează efectele benefice ale produsului. Sunt analizate 4 vinuri albe și unul roșu din oferta comercială. Rezultatele variază în limite largi dar sunt mai mari decât unele observații de la microproblele de laborator.*
Cuvinte cheie: *vin comercial, DPPH, chemiluminiscență, compuși fenolici*

INTRODUCTION

Radicals are defined as atoms, compounds, or ions that contain an unpaired electron in their atomic or molecular orbital. Free radicals or free species are well recognized for having both negative and beneficial effects on living systems. From those oxygen free radicals, commonly referred to as reactive oxygen species (ROS), but in addition to these are also reactive nitrogen species (RNS) generated at low/moderate concentrations. They are important in numerous physiological functions including: regulation of vascular tone, sensing of oxygen tension, enhancement of

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signal transduction from various membranes, and oxidative stress responses that ensure the maintenance of redox homeostasis (Halliwell and Gutteridge, 1989).

The evolution of normal metabolic processes such as respiration and photosynthesis unavoidably leads to the production of free radicals. In contrast, an overproduction of ROS can cause damage to cellular lipids, proteins, or DNA, inhibiting their normal function. However, organisms have evolved a complex array of defences comprising of both endogenous (enzymatic and non-enzymatic) and exogenous antioxidants to control cellular levels of free radicals (Jaeschke, 2010). These species have been recognised to protect cells by scavenging free radicals from the body (causing a breakdown of radical chain reactions), suppressing free radical formation or by reducing oxidised cellular components (Valko *et al.,* 2007).

The methods develop are to evaluate the antioxidant capacity of whole plants other foods. Traditionally these are often referred to as off-line or batch style approaches as they are generally performed in spectroscopic cuvettes. Two of the more commonly used off-line techniques are based on measuring the ability of exogenous antioxidants to reduce a stable radical (representative of in vivo free radicals). The first approach involves the purple chromophore radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH°) which is reduced by an antioxidant(s) to its corresponding pale yellow hydrazine. This can be spectrophotometrically monitored as a decrease in absorbance at 515-517 nm. After a period of time, the degree of decolourisation and decrease in absorbance is proportional to the number of electrons captured, and therefore to the concentration and antioxidant activity of the species itself. The results of this technique are generally reported as the ED50, defined as the amount of antioxidant necessary to decrease the initial DPPH° concentration by 50% (Brand-Williams *et al.,* 1995).

MATERIAL AND METHOD

In this study we are examining the possibly to improve radical scavenging determination so the number of sample is reduced at four white wine (Huși Averești Zghihară 2013; Cotnari: Fetească albă 2011, Fetească albă 2013 and Tămâioasă românească 2011) and one red wine, Fetească neagră Dealu Mare year of production 2013, all commerciale available on romanian market. For avoiding any kind of legal problem we are just mention the grape sort, region and year of production.

The platform for the antiradical depletion DPPH is made with a Visible or UV-Vis spectrophotometer,in our case multi-plate reader Tecan M200 Pro (fig. 1) withpolymethyl methacrylate well plates. A series of dilution is needed so it can calculate the final result. This dilution are: Reference solution D_0: 9 mL of the DPPH° methanolic solution + 100 µL of methanolMeOH; dilution D_1: 1/40 dilution of wine (4 mL of the DPPH + 100 µL wine); dilution D_2: 1/80 dilution of wine (4 mL DPPH° + 50 µL wine); D_3: 1/160 dilution of wine (4 mL DPPH° + 25 µL wine); D_4: 1/320 dilution of wine (4 mL DPPH° + 12.5 µL wine); D_5:1/640 dilution of wine (4 mL DPPH° + 6.25 µL wine).

The antioxidant activities is evaluated based on free DPPH° radicals remaining in the medium after the reaction between the methanolic DPPH° solution and the samples to be tested.For each dilution from $D_0$to D_5, the reduction in the absorbance is determined at 515 nm at 0 min and every 1 min. for 14 min., and every 10 min. until the reaction reaches

a plateau in about 1 hour. In some cases the percentage of DPPH at the plateau level can be calculated: DPPH° remaining in a stable state (%) = [(Ci - Cf) / Ci] × 100. The antioxidant activity of the wine is thus defined by the dilution of wine required to decrease the initial concentration of DPPH° by 50%: Effective dilution = ED50.

Fig. 1 - Multi-plate reader used for the determination of the DPPH scavenging activity

Determination of water-soluble and lipid-soluble antioxidative capacity (ACW and ACL) in wine is realised using the methodology put in place by the Analytik Jena company (***, internet). The reaction kits are conserve to freezer until needed. The reaction uses luminoland the activation is done inside the Photochem device by a high intensity UV lamp. The results are in nmol/L of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid for the ACL determination) and nmol/L ascorbic acid for ACW.

We have used as a platform we developed a method presented in Journal of Chromatography A. (Castellari M. et al., 2008). For the analysis of phenolic compounds, samples were processed on a Shimadzu HPLC consisting of: quaternary pump Shimadzu Prominence series LC-20AD with five-channel degasser DGU-20A5 Shimadzu Prominence series, autoinjector SIL-20AC HT Shimadzu Prominence series (injection volume: 10 µL, sample temperature 20 °C), column oven CTO-20AC Shimadzu Prominence series, diode array detector SPD-M30A Shimadzu Prominence series (190-600 nm), chromatographic system controller CBM-20A Shimadzu Prominence series PC connectivity via LAN. We optimized the gradient using trifluoroacetic acid (TFA) as an eluent acidification of 1% MeOH (A channel) and 50% MeOH (B channel) acidified to 2.15 pH with TFA. The column system is composed of a pre-column SecurityGuard ULTRA Cartridges UHPLC C18 for 4.6 mm ID coupletto Kinetex 2.6 µm C18 100Å 150×4.6 mm columns manufactured by Phenomenex. The elution flow is 0.85 mL/min and the column compartment is set at 50 °C so we can make a total run in 60 min.

RESULTS AND DISCUSSIONS

In the past we implemented the classical methodology in a Uv-Vis spectrometer. The problem with this kind of determination is related to the dilution of the sample. If the radical scavenging activity is greater than the concentration on DPPH is methanolic solution, the reaction is fast under 2-3 minutes and you can miss the initial concentration or the start of the reaction. So from our experience the manipulation and shaking for mixing the reactance is more or less taking 1 maybe 2 minute. In order to avoid this kind of problems we

began to experiment, with multi-plate readers. In this study the adding and mixing is done automatically, but still another problem appeared at low volumes the dominant substance MeOH begins to evaporate during the reaction, so the number of repetition are at more than 9 in order to get reproducible results.

In figure 2 it's presented the effect of concentration of red wine upon different dilution in 5 min from start of the reaction. The other figure presents the instability of this solution in time as result of wine matrix (the lowest dilution).

Fig. 2 - Consumption of methanolic solution of DPPH radical by colour change (from purple to yellow) in the five dilution (D_1, D_2, D_3, D_4 and D_5) compared to the control D_0

Fig. 3 - Consumption of methanolic solution of DPPH radical, by colour changing of the diluted sample (from purple to yellow) in a single dilution (D5) for 60 minutes in air

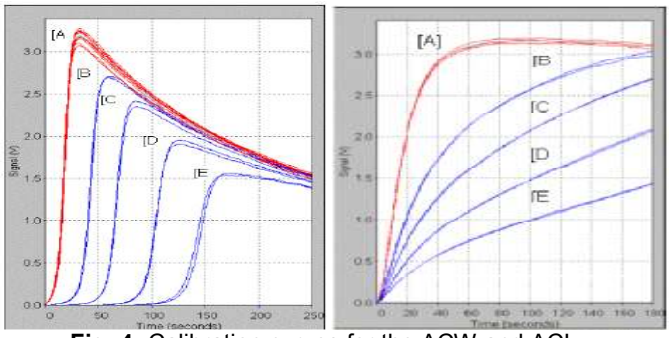

Fig. 4 -Calibration curves for the ACW and ACL

The analysis wine with the PhotoChem device is more robust and reproducible (n=3). In the figure 4 are presented the different types of kinetics involved in the foto-induced radical formation.

Table 1

Wine anti-scavenging activity

WINES Grape sort Region/Producer Year of production	DPPH ED50	Ascorbic acid equivalents [mmol/l]	Trolox equivalents [mmol/l]
ZghihărăAverești 2013	1/35	1.84	0.28
FeteascăalbăCotnari 2011	1/18	1.37	0.19
FeteascăalbăCotnari 2013	1/23	1.38	0.22
TămâioasăromâneascăCotnari 2011	1/58	1.41	0.27
FeteascăneagrăDealu Mare 2013	1/324	3.01	0.52

Table 2

Phenolic compound quantified in wines

mg/L	Zghihară Averești 2013	Fetească albă Cotnari 2011	Fetească albă Cotnari 2013	Tămâioasă românească Cotnari 2011	Fetească neagră Dealu Mare 2013
gallic acid	272.63	439.74	173.38	675.59	6315.54
protocatechuic acid	33.28	28.38	22.62	59.13	79.41
p-hydroxybenzoic acid	946.15	293.65	329.32	676.87	816.66
gentisic acid	16346.53	5070.44	5740.75	11506.64	13686.39
B1	11.31	42.34	64.36	49.19	123.11
catechin	25.29	28.20	32.39	69.03	75.63
m-hidroxybenzoic acid	29.27	182	95.53	66.43	2307.29
vanillic acid	36.65	48.21	59.12	40.86	594.48
caffeic acid	115.87	491.33	66.36	264.33	562.87
chlorogenic acid	1.5	4.83	2.88	5.03	28.73
B2	1.85	1.61	1.39	3.45	57.75
syringic acid	1.63	6.24	3.93	5.32	211.1
epicathechin	0.46	36.6	1.23	33.98	132.85
p-coumaric acid	21.71	100.98	24.91	68.04	201.71
ferulic acid	46.03	248.94	249.85	129.03	45.68
salicylic acid	9.13	18.63	108.05	4.16	60.53
polydatine	0.12	0.04	0.03	0.31	7.45
sinapinic acid	1.54	0.45	0.62	0.29	0.71
hyperoside	0.59	0.04	0.04	0.07	0.34
ellagic acid	0.05	-	-	0.06	2.89
trans-resveratrol	12.45	10.28	7.6	11.73	471.71
rutin	11.55	7.13	13.4	9.39	32.22
cis-resveratrol	0.31	2.89	0.64	0.74	0.96
morin	0.12	0.49	0.35	0.45	0.08
quercitine	1.18	7.33	1.62	9.57	3.54
∑ eq./L	116.45	44.6	44.76	87.81	161.97

In table 1 the antiradical activity of red wine is overwhelming up to 9 time greater than white wine and also the maceration of Tămâioasa is not a factor of improvement. The age of the wine are diminishing the activity. The acidity of the Zghihara wine could have some effect on the ACW determination. In case of wine the ACL values are not to be correlated with the lipophilic compound's, but mainly with the complexity of water non soluble phenolic substances present in wine.

The phenolic composition in table 2 show haw complex is the composition of different wine. Each winehas a different phenolic composition and this reflects the maceration or the technology specific for different region or people, but the total results concurs with bioactive proprieties determined.

CONCLUSIONS

1. The improved method for evaluating antiradical capacity enables more stable analysis with DPPH stable radical.

2. The antiradical ability of white wines commercially available is proportional to the amount of phenolic compounds detected whit LC method.

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