ANALELE UNIVERSITATII DIN ORADEA
FASCICULA: ECOTOXICOLOGIE, ZOOTEHNIE SI TEHNOLOGII DE INDUSTRIE ALIMENTARA, VOL. VII, ANUL 7, 2008

DETERMINATION OF ANTIOXIDANT ACTIVITY OF THE PHENOLIC COMPOUNDS FROM GRAPE POMACE

Simona Bisboaca*, Cornelia Purcarea**

*University of Agriculture and Veterinary medicine Cluj-Napoca – Faculty of Veterinary medicine – Department of Pharmacology and Toxicology arleena@gmail.com
**University of Oradea – Faculty of Environmental Protection University of Oradea – Faculty of Environmental Protection neli_oradea@yahoo.com

Abstract

Lately medical scientific research has been focused on the non alcoholic compounds from wine, especially phenolic compounds. These compounds have remarkable health promoting properties, cardio-vascular protective, with anticarcinogenic, antiviral, antihistaminic, anti-inflammatory even antiHIV action. Plant tissues contain a great variety of hydroxylate flavonoids and other phenolic compounds from traces to grams/kg. Grapes have a high concentration of phenolics and a part of them pass into the wine and the rest remain in the pomace after the wine making. This study is referred at these phenols, how to valuate them and study their antioxidant activity. A part of the phenolic compounds have a good antioxidant activity, some inhibits LDL in vitro. Recapture the phenols from pomace is considered an advantage and have a large utilization from food industry, to pharmaceutical and cosmetics.

Key words: grapes, phenols, antioxidant capacity.

INTRODUCTION

Phenolic compounds are widely distributed in plant foods and therefore important constituents of the human diet. Phenolic compounds, at low concentration, may act as an antioxidant and protect foods from oxidative deterioration. However at high concentrations, they may interact with proteins, carbohydrates and minerals. Phenolic compounds are important by their contribution to human health with their multiple biological effects such as antioxidant activity, antimutagenic and/or anticarcinogenic activities, and anti-inflammatory action. In recent years, the numbers of studies which are conducted to determine antioxidant activity of phenolic compounds have increased due to the possible role of reactive oxygen species in the pathogenesis of degenerative diseases such as atherosclerosis, cancer and chronic inflammation. Antioxidant activities of phenolic compounds have been intensively studied using *in vitro* methods. The results of these studies showed that phenolic compounds are powerful antioxidants.

Phenolic compounds in grapes are accumutated in the solid parts like seeds, bunch, skin. From here, after technological process of marc maceration-fermentation the compounds pass in the must, but a good percentage remain in the pomace. The percentaje of their solubilisation in

the must is in relation with the wine making technology that is used. The phenolic compounds from grapes gives to the must and wine the color, corpulence, astringency, flavour, vitamins, participate at oxidoreduction, condensation reactions, have bactericide properties. The total content of phenolics in grapes varies from 2.9 g/kg to 5.7 g/kg (4).

The aim of the study was to determine the antioxidant activities of grape pomace dried at sun light, dried at dark and frozen.

MATERIALS AND METHODS

The rough material, Muscat white grape pomace from Santimbreu, Bihor County was selected for this study. The grapes were hand picked at full maturity, 230 g sugar/l. It has been used the classical white wine making method. 1500g of selected pomace was divided in 3 parts for storage. First assay was freezed – method used to inactivate polyphenol oxidase. The second assay was light dried at 25°C and the third assay was dried at dark at 25°C.

The assays were marked as follow:

- um – freezed assay (humid)
- us hν - light dried assay
- us – dried at dark

All assays were grindined and triturated to eliminate the rough parts (like lignified bunch) and to crash the seeds to speed up the extraction. Measure from each assay 6 grams and pour over 20ml of ethanol 70%. Use the same extraction method with 6 grams of each assay and pour 20 ml of absolute methanol. To speed up the extraction all assays were incubated on bain marine at 60 °C for 15-20 minutes. After the extraction filter the three assays. The filtration is made with paper filter until all mucilage is removed. The clear liquid is read at spectrophotometer.

The extracts described above were used to register the spectroscopic print.

For reading the absorption of each assay make the follow dilutions:

1. freezed assay – ethanol extraction (um 1) – dilution 1:5 meaning 1 ml extract with 5 ml solvent (ethanol 70%).
2. light dried assay – ethanol extraction (us hυ 1) – dilution 1:10 meaning 1 ml extract with 10 ml solvent (ethanol 70%).
3. dark dried assay – ethanol extraction (us 1) – dilution 1:10 meaning 1 ml extract with 10 ml solvent (ethanol 70%).
4. freezed assay – methanol extraction (um 2) – dilution 1:5 meaning 1 ml extract with 5 ml solvent (absolute methanol).
5. light dried assay – methanol extraction (us hυ 2) – dilution 1:5 meaning 1 ml extract with 5 ml solvent (absolute methanol).

6. dark dried assay – methanol extraction (us 2) – dilution 1:5 meaning 1 ml extract with 5 ml solvent (absolute methanol).

Spectrum UV-VIS was registered on a range from 200nm to 600 nm, with a Perkin Elmer, Lambda 3.

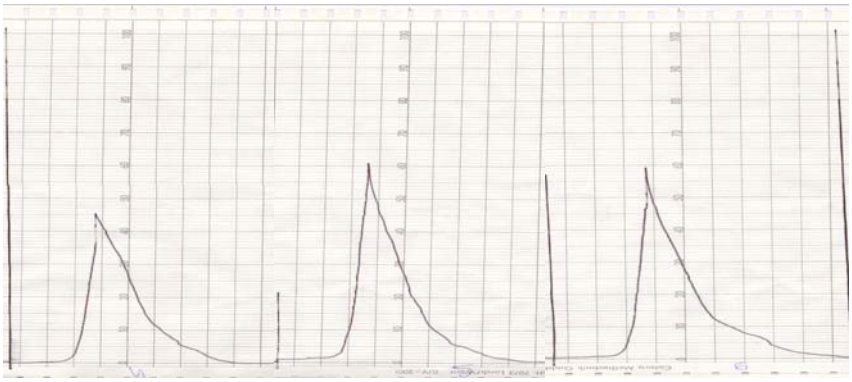
The antioxidant activity is determined with TBARS method. Prepare TBARS reagent with 30 g of tricloracetic acid, 0,75 g of thiobarbituric acid and 5,2 ml of HCl (0,25 N) and bring to sign until 200 ml with distillated water. In each test tube insert 100 µl ethanolic extract assay (um1, us1, us hυ1) add 900 µl Tris tampon and 2 ml MDA reagent, vortex for a few seconds then boil 15 minutes. Empty the content in centrifugal test tubes, centrifuge 10 minutes at 1000g. Read the extinction at λ_{max}= 535 nm (DO_{535}). Use the following method to calculate the number of MDA mols formed in the reaction:

$$\text{mmol MDA/100 µl assay} = DO_{535} \times 1{,}56 \times 105$$

To induce the per oxidation use sunflower oil mixed with H_2O_2 3%, (100 µl) then measure DO_{535} for each assay (1).

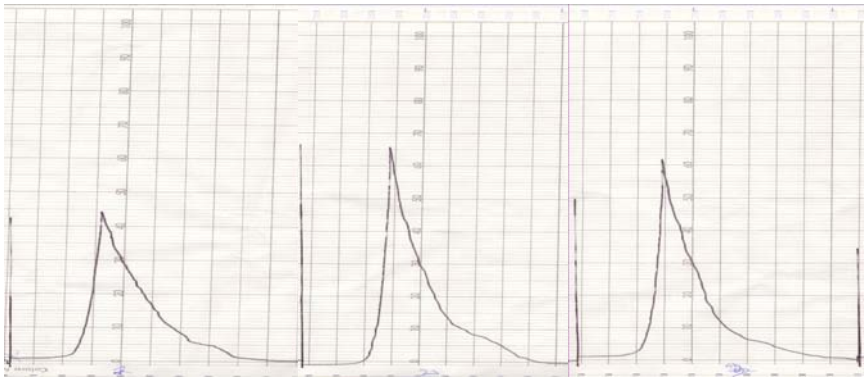
RESULTS AND DISCUSSION

Figures 1-2 represents the spectrophotometer prints of the *um*, *us hν* and *us* assays registered after the ethanolic and methanolic extraction. Based on these specters were identified the wave length representative for the phenolic compounds (flavonoids 320 – 355 nm) and values of the absorption at λmax:



um 1 us 1 us hν 1

Fig.1 UV-VIS spectrum for ethanolic extraction



um2 us 2 us hν 2

Fig.2 UV-VIS spectrum for methanolic extraction

Table 1

№	Assay	Wave length λmax [nm]	Absorption (DO)
1	um 1	329	0,450
2	us hυ 1	334	0,610
3	us 1	335	0,590
4	um 2	322	0,440
5	us hυ 2	327	0,620
6	us 2	328	0,660

It has been used a stamp method with quercetol as a standard. To valuate the relation concentration – absorption in the analyze assays at λ max 330 nm (tabel 1)were used growing concentrations of quercetol on the rage 1-5 mg/100ml. In based of the calibration curve was determined the factor f = 35,6 and the flavonoids concentartion were calculate in tabel 2.

Table 2.

Assay	$DO_{\lambda\ max}$ [nm]	Concentration [mg/100 g] material dry subst.
um 1	0,450	51,67
um 2	0,440	50,52
us 1	0,590	43,31
us 2	0,660	24,22
us hν 1	0,610	45,22
us hν 2	0,620	22,99

The evaluation of the antioxidant property of the ethanolic and methanolic extracts

Below, in Table 3 are represented the DO_{535} values and the quantity of MDA resulted after the incubation of the assays with and without per oxidation inductor.

Table3.

Nr.	Assay [µl]	Tampon [µl]	MDA [ml]	Oil + H_2O_2 3%[µl]	Absorption (DO 535 nm)	mmol MDA / 100 µl assay	Obs.
1	-	900	2	100	Etalon	0	Control sample for 6-11
2	-	1000	2	-	Etalon	0	Control sample for 3,4,5
3	um 1 / 100	900	2	-	0,240	39,312	
4	us 1 / 100	900	2	-	0,610	99,918	
5	us hν 1/ 100	900	2	-	0,520	85,176	
6	um 1 / 100	800	2	100	0,435	71,253	
7	us 1 / 100	800	2	100	0,640	104,832	
8	us hν 1/ 100	800	2	100	0,580	95,004	
9	um 1 / 200	700	2	100	0,610	99,918	
10	us 1 / 200	700	2	100	0,790	129,402	
11	us hν 1/ 200	700	2	100	0,910	149,058	

Antioxidant capacity (AC) of the assays was determined by subtracting the assays incubate with the mix of oil and $H_2O_2$and the assays without inductor. In Table 4. it's represented the AC after this calculation method:

$AC_{100} = 100 \times (PO_{I\,100} - PO.) / PO_{I\,100}$ - when used 100 µl extract

PO – peroxide (determined with TBARS)

$AC_{200} = 100 \times (PO_{I\,200} - 2xPO.) / PO_{I\,200}$ - when used 200 µl extract

PO – peroxide (determined with TBARS)

The value of parameter *2* from the formula represents the subtract of the extract dilution.

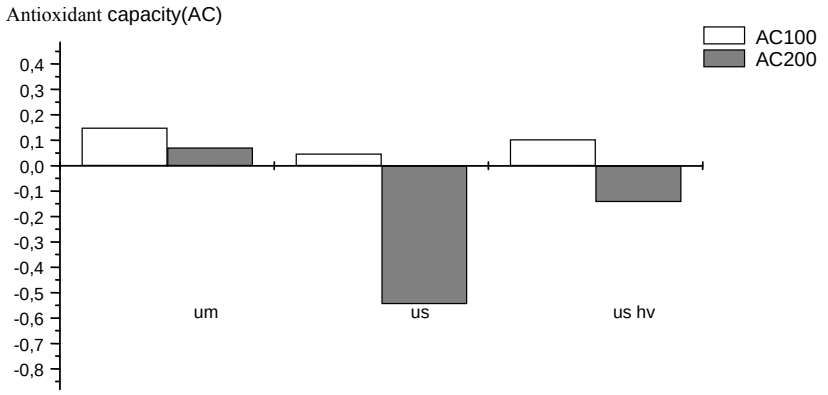
Table 4.

Assay	**AC_{100}**	**AC_{200}**
um	0,448	0,213
us	0,046	- 0,544
us hν	0,103	- 0,142

CONCLUSIONS

The negative values indicates a strong antioxidant efect of the extract towards the per oxidation mix inductor, in the mean time the positive values indicates a decrease of the antioxidant efect. The majority of the phenolic compounds(flavonoids with the peak between 320 -355 nm.) that were identified waves from 21,99 to 51,67 mg/ 100 g of dry material. The highest concentrations were observed when the extraction was made with methanol.

ANTIOXIDANT CAPACITY OF PHENOLIC EXTRACTS FROM GRAPE POMACE



From the investigated assays, the one dried at dark has the highest value of the AC while the frozen assay was less active. One simple explanation is that during the processing of the assay the enzyme polyphenol oxidase was activated and used the flavonoids as a substrate and so the concentration of phenols is decreasing. Drying at dark it's proved to be a better storage method to preserve the phenols and their antioxidant activity.

REFERENCES

1. Frankel E.N., J.Kanner, E.German, J.Parks, 1993, Inhibition of human LDL by phenolic substances, The Lancet, nr. 341.
2. Masquelier J. , 1982, Vin et athérosclerose. Action protéctive des procyanidines, Symp.Intl. Alimentation et Consommation du Vin – Ed. Fondazione Sergio Bola, Verona.
3. Renaud S., M. Largeril, 1992, Wine, alcohol, platelets and the franch paradox for coronary heart disease, The Lancet, nr. 339.
4. Târdea C., A.Târdea, 2001, Tratat de vinificaţie, ed. Ceres.
5. Vrhovsik U., S. Wendelin– Influence of winemaking, technologies, storage and fining on the balance of white wine hydroxycinnamates – Instituto Agrario SanMichele