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RESEARCH ARTICLE

THE EVALUATION OF THE WINES ANTIOXIDANT ACTIVITY

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Abstract

A new method for measuring the antioxidant activity is the method which using N, N' –dimethyl-p-phenylendiamina (DMPD). The method is rapid and inexpensive, ensures sensitive and reproducibility. In this paper, was verified of there effectiveness of the DMPD method on antioxidant foods. We used wine samples coming from different areas of Romania. Antioxidant action of wines is strictly related to the amount of phenolic compounds. To evaluate the sensitivity of the method, the system was tested by using of standard solution of TROLOX 1mg/mL and DMPD: $FeCl_3$ molar ratio of 10:1. Spectrofotometric measurements were recorded by using an UV-VIS Jenway 6300 at 505 nm. Antioxidant action was expressed as TEAC (TROLOX equivalent antioxidant capacity), using the calibration curves plated with different amounts of TROLOX. These results show that the red wine samples have a high antioxidant action, in conformed to the amount of phenolic compounds. The method ensures sensibility and reproducibility in the measurement of antioxidant action of hydrolytic compounds.

Key works: wines, DMPD method, Trolox

Introduction

Cancer is a leading cause of death and may result from chronic injury to the epithelium by oxidants and other carcinogens (Murphy, 1997). Epidemiological and experimental studies also offer strong evidence that implicates oxidative damage in the etiology of brain, heart and nervous system diseases (Yoshikawa *et al.*, 1997). Although the body has effective defence systems that protect it against oxidative stress, the capacity of these protective systems decreases with aging creating a need to provide the body with a constant supply of phytochemicals through dietary supplements (Halliwell *et al.*, 1999). French people include in the daily diet a glass of red wine and this way, the cardiovascular accidents are 2,5 less than at the American consumers of alcoholic drinks (Buyukokuroglu *et al.*, 2001).

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The analysis of the composition of wine demonstrated that it contains over 1000 benefic substances for the organism. Among the most important are the phenols, carbohydrates, mineral elements (K^+, Ca^{2+}, Mg^{2+}), vitamins (A, B_2, B_5, B_6, C), organic acids, compound aromatics and proteins (Tinttunen *et al.*, 2001). The phenols are found in a higher quantity in red wines (3-5 g/L) than in the white ones. Because of their antioxidant action, the phenols from the wine annihilate the negative action of the free radicals, stopping the early aging and degenerative illnesses (Lopez *et al.,* 2003).

The antioxidant protection is ensured by SO_2, which is used and accepted in all the countries for its multiple actions, amongst which we mention (Pellegrini *et al*., 2000) : the antiseptic action, the action of inhibition of the enzymatic activity by blocking the activity of the complex of oxidative enzymes (polyphenoxidase, peroxidase and ascorbicoxidase). SO_2 are the action of reduction of the pH value and in this way, the solvability of the antocianes, the application of stabilization treatments and the increase of the antimicrobial efficiency are facilitated. Romania is an important European country that produces wine, having an important historic past and rich cultural tradition, many of them related to viticulture.

Nowadays, the country is in a period of great changes, building a future in European Union and aspirates o become an appreciated member of the international community of the wine as producer of high quality wines.

A lot of the autochthon are known (White Feteasca, Black Feteasca, Yellow of Odobeşti), as well as an impressive number of 20-30 wines imported from foreign countries (Chardonnay, Sauvignon, Aligoté for white of wines; Cabernet Sauvignon Merlot, Pinot Noir for red wines). On the map of Romania are eight wine regions where the wine cultures are grouped in 38 podgorias with a total of 123 viticol centers and 40 independent viticol centers (Gheorghita *et al.*, 2002).

The researches made until now suggest that the Romanian wines present benefic vasodilators and ant sclerotic qualities, similar to those that stay at the base of the so called "French paradox" [9].

In this context, in this paper it has been followed the antioxidant action of different Romanian and Italian wines-antioxidant action sustained by the antioxidant compounds of the wines - the phenols, as well as "active SO_2" which is formed during keeping of the wines.

Materials and methods

Chemicals Reagents. Folin-Ciocalteu phenol reagent, tanic acid, anhydrous sodium carbonate, anhydrous ferric chloride were purchased from Sigma Chemical Company; *N,N*-Dimethyl-*p*-phenylenediamine dihydrochloride (DMPD) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) were purchased from Aldrich, Germany; all solvents (methanol) and reagents (deionized water; acetate buffer pH 7, iodine) were purchased from local suplliers.

Samples of wine. Nine red (1993-1996) and eight white (1996-1998) wine samples originating from different areas of Dobrogea, Romania and areas of Compania and Sicilia, Italia, were purchased from local and Italian markets.

Apparatus. Spectrophotometer measurements were recorded by using an UV-VIS Jenway 6300 apparatus.

Total Phenolic Content of Wine Samples. The phenolic content of the different wines was determined by Folin-Ciocalteu reagent (Meir *et al.*, 1995). Each sample (0.1 mL) was added to 4.2 mL of deionized water and 0.5 mL of Folin-Ciocalteu reagent (Sigma). After 1 min of mixing, 1 mL of an 80% solution of sodium carbonate and 4.2 mL of deionized water were added. The mixture was left 2 h at room temperature in the dark and the absorbance at 760 nm was measured. The concentration of the total phenolic content was determined by a comparison with the values obtained with a standard solution of tanic acid (0,01%).

The total content of phenolic compounds in the extract in tanic acid equivalents was calculated by the following formula = CxV_1/V, where: T = total content of phenolic compounds, µg/mL wine, in tanic acid; C = the concentration of tanic acid established from the calibration curve (µg/mL);

V = the volume of wine sample, milliliter; V_1 = the volum of product (1mL wine). *Sulfur Dioxide Determination***.** Total and free SO_2 content of wine samples was determined by the titrimetic method „Ripper" ["personal communications"] using solution of iodine 0.1N. *Scavenging Effect (%) by* DMPD method (Vincenzo *et al.*, 1999). DMPD, 100 mM, was prepared by dissolving 209 mg of DMPD in 10 mL of deionized water; 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer, pH 5.25, and the colored radical cation ($DMPD^{\cdot+}$) was obtained by adding 0.2 mL of a solution of 0.05 M ferric chloride (final concentration 0.1 mM). One milliliter of this solution was directly placed in a 1-mL plastic cuvette and its absorbance at 505 nm was measured. Standard solutions of the TROLOX were prepared as follows: 1mg/mL of TROLOX was prepared by dissolving 0.1 g of TROLOX in 100 mL of methanol. Fifty microliters of standard antioxidants or of wine samples (diluted in water 1:20 for the red wines, undiluted for white wines) were added in the spectrometric cuvette and after 10 min at 25 °C under continuous stirring the absorbance at 505 nm was measured. The buffered solution was placed in the reference cuvette.

A dose-response curve was derived for TROLOX, by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution (blank) according to the equation:

$$\text{inhibition of } A_{505}\ (\%) = \left(1 - \frac{A_f}{A_0}\right) \text{x} 100$$

where: A_0 is the absorbance of uninhibited radical cation and A_f is the absorbance measured 10 min after the addition of antioxidant samples. Antioxidant ability of fish oil was expressed as TEAC (TROLOX equivalent antioxidant capacity) according to DMPD method, using the calibration curve plotted with different amounts of TROLOX.

Statistical Analysis. All data were expressed as mean ± SD (n=3) by using *Origin 8* test. Mean values do not differ significantly.

Results and discussions

Wine was widely studied for its antioxidative properties due to the wellknown health importance of its phenolic component.

Antioxidant compounds in wine are mainly hydrophilic and their antioxidant activity could be well evaluated by the DMPD method.

Total Phenolic Content and Sulfur Dioxide of Wine Samples

The 17 wine samples were tested for their antioxidant ability. The concentration of the total phenolic content was determined by using calibrasion curve of tanic acid (see Fig.1). The standard deviation is very low and the dose – response curve is highly reproductible.

The ecuation of calibration curve is:

C = 11,038 A – 0,269; Y = A * X; A = 0.122033;
Correlation Coefficient = 0.99519;
Standard Error = 0.665321;
r = 0.99519; r^2=0,99040.

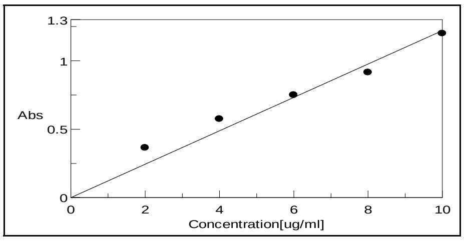

Figure 1. *Calibration curve for tanic acid*

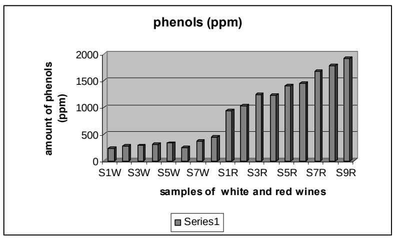

Figure 2. *The amount of phenols in sample of wines*

Table 1. *Amounts of polyphenols of tested wines*

White wines			Red wines		
Samples	**Type of wine**	**Amount of polyphenols (ppm tanic acid)**	**Samples**	**Type of wine**	**Amount of polyphenols (ppm tanic acid)**
S_1	C.*	288	S_1	C.S_1	1780
S_2	M.$_1$.*	240	S_2	C. S. $_2$	1400
S_3	M.$_2$	378	S_3	M_1*	1230
S_4	S_1	312	S_4	M $_2$	1445
S_5	S_2	332	S_5	B.	1920
S_6	R_1	255	S_6	P. N.$_1$	1675
S_7	R $_2$	281	S_7	P. N_2	1245
S_8	R $_3$	445	S_8	B.*	935
			S_9	C.	1025

*On the label is mentioned "it contains sulfites"

The content of phenols are indicated in Table 1, respectivelly in Fig. 2, Fig. 3 and Fig.4.; the total and free SO_2 are indicated in Table 2 and 3.

The present study shows the presence of the phenols in higher quantity in the red wines 900-1900 (ppm of tanic acid), than in the white ones 200-450 (ppm of tanic acid).

The obtained data are in concordance with the speciality literature.

This way, on the basis of the contain of polyphenols, the data from literature shows the miorelaxing and vasodilating action more intense in the case of the red wines than in the white ones; some of the red wines contain 1,72-1,91g/L and the white ones contain between 0,43 and 0,46 g/L.

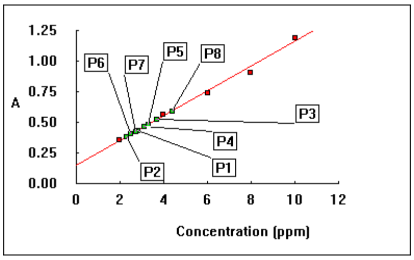

Figure 3. *Amounts of phenols of tested white wines*

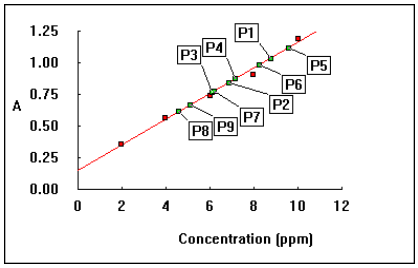

Figure 4. *Amounts of phenols of tested red wines*

The results are sustained by the content of total SO_2 which at the white wines is higher 70-188 ppm than at the red ones 46-90 ppm and higher at the types which have mentioned on the label "it contains sulfites".

Regarding to the SO_2 analysis, the maximum admitted quantity is not higher in any of the samples, value registered by O.M.S. 975/1998 (Order of Health Minister) and C.E. (European Commission). Staying at the same quantity of SO_2 allows us to sustain that adding the preservative does not have the risk of modification the organoleptic and nutritive value of the product. The obtained results are given in Tables 2 and 3 and their analysis is made in according with the values accepted by O.M.S. 975/1998. This way:- the accepted quantity of total SO_2 in wines is: 160mg/L for the red wines with small quantity of carbohydrates; 260 mg/L for the white wines with small quantity of carbohydrates; 300mg/L for the wines with higher quantity of carbohydrates.-the quantity of free SO_2 accepted in wines is 50 mg/L.

***Table 2.**- Amounts of free SO_2 of tested wines*

Samples	Type of wines	Free SO_2 ppm	Standard Deviation	Confidence Intervals for Mean 95% Minimum	Confidence Intervals for Mean 95% Maximum
			White wines		
S_1	C.*	25,39	0,190	25,23	25,60
S_2	$M._1$.*	23,46	0,344	23,24	23,86
S_3	$M._2$	19,25	0,055	19,20	19,31
S_4	S_1	7,61	0,061	7,56	7,68
S_5	S_2	0,51	0,023	0,49	0,54
S_6	R_1	0,70	0,011	0,69	0,71
S_7	R_2	0,92	0,020	0,9	0,94
S_8	R_3	1,41	0,100	1,34	1,53
			Red wines		
S_1	$C.S_1$	3,55	0,092	3,45	3,63
S_2	C. S. $_2$	2,83	0,050	2,79	2,89
S_3	M_1*	7,23	0,196	7,01	7,36
S_4	M_2	8,29	0,070	8,21	8,34
S_5	B.	6,99	0,064	6,92	7,04
S_6	P. $N._1$	4,47	0,017	4,45	4,48
S_7	P. N_2	0,69	0,078	0,64	0,78
S_8	B.*	10,3	0,0721	10,24	10,38
S_9	C.	9,10	0,0832	9,04	9,20

Each value is a mean of triplicate analyses ±SD. Mean values do not differ significantly.

*On the label is mentioned “it contains sulfites”.

***Table 3**. Amounts of total SO2 of tested wines*

Samples	Type of wines	Total SO_2 ppm	Standard Deviation	Confidence Intervals for Mean 95% Minimum	Confidence Intervals for Mean 95% Maximum
			White wines		
S_1	C.*	187,95	0,155	187,78	188,08
S_2	$M._1$.*	182,27	0,328	181,9	182,5
S_3	$M._2$	146,32	0,372	146	146,73
S_4	S_1	130,27	0,266	130,03	130,56
S_5	S_2	138,07	0,176	137,89	138,24
S_6	R_1	110,16	0,141	110	110,25
S_7	R_2	90,18	0, 060	90,13	90,25
S_8	R_3	76,51	0,260	76,29	76,8
			Red wines		
S_1	$C.S_1$	85,61	0,167	85,43	85,76
S_2	C. S. $_2$	90,76	0,196	90,54	90,88
S_3	M_1*	76,60	0,309	76,25	76,8
S_4	M_2	87,02	0,055	86,99	87,09
S_5	B.	85,63	0,107	85,56	85,76
S_6	P. $N._1$	90,73	0,174	90,54	90,88
S_7	P. N_2	46,07	0,055	46,02	46,13
S_8	B.*	71,70	0,115	71,57	71,78
S_9	C.	87,02	0,130	86,89	87,15

Each value is a mean of triplicate analyses ±SD. Mean values do not differ significantly.

*On the label is mentioned “it contains sulfites”.

Table 4. *The ratio between the form of SO_2*

Samples	Free of SO_2		Combinated of SO_2		Total of SO_2
	ppm	%	ppm	%	ppm
White wines					
S_1	25,39	13.60	152,40	86,40	188,00
S_2	23,46	14,70	125,92	85,30	159,21
S_3	19,25	15,11	117,68	94,89	126,88
S_4	7,61	6,23	103,68	93,77	122,88
S_5	0,51	0,36	137,20	99,64	137,72
S_6	0,70	0,64	108,84	99,36	109,56
S_7	0,92	1,04	88,39	98,64	89,32
S_8	1,41	1,90	74,14	98,10	75,52
Red wines					
S_1	3,55	15,22	82,12	84,78	85,76
S_2	2,83	18,08	87,79	81,82	90,88
S_3	7,23	5,40	69,44	94,60	76,80
S_4	8,29	10,26	78,72	89,74	87,04
S_5	6,99	27,05	78,66	**72,85**	85,76
S_6	4,47	20,40	86,40	81,47	90,88
S_7	0,69	8,68	45,44	91,32	46,08
S_8	10,3	14,30	61,52	85,70	71,76
S_9	9,10	10,30	79,00	91,70	87,04

Comparing the values of the SO_2 total/combined from the white wines with its value from the red ones is observed that the assortments if white wines have more total/ combined SO_2, especially those which have mentioned on the label "it contains sulfites" – Table 4. The percentage of the "free active form "is very small, even sub unitary at some assortments; in the first three samples of white wines is found also in relatively small percentage 13%-15% for ensuring an antioxidant protection. Normally, the free SO_2 represents 15%-30% from the total SO_2, but the antiseptic and antioxidant actions have only 2%-10% free SO_2 (Tirdea *et al.*, 2000).

From the ratio between the quantity of free SO_2 and total SO_2 (Table 4) is observed that not always the higher value of total SO_2 means a higher percentage of "active dioxide", which shows that the sulphitation process is complex and has unexpected final effects.

The antioxidant ability of wine samples

The principle of the assay is that at an acidic pH and in the presence of a suitable oxidant solution DMPD can form a stable and colored radical cation ($DMPD^{\cdot+}$) (Scheme 1, step 1). The UV-visible spectrum of $DMPD^{\cdot+}$, reported in Fig.5, shows a maximum of absorbance at 505 nm (Vincenzo *et al.*, 1999). Antioxidant compounds (AO) which are able to transfer a hydrogen atom to $DMPD^{\cdot+}$ quench the color and produce a decoloration of the solution which is proportional to their amount (Scheme 1, step 2). This reaction is rapid (less than 10 min) and the end point, which is stable, is taken as a measure of the antioxidative efficiency. Results are reported in Table 5.

$$DMPD(uncolored) + oxidant\ (Fe^{3+)} + H^{+} \rightarrow DMPD^{\cdot+}\ (purple)$$

$$DMPD^{\cdot+}\ (purple) + AOH \rightarrow DMPD^{+}\ (uncolored) + AO$$

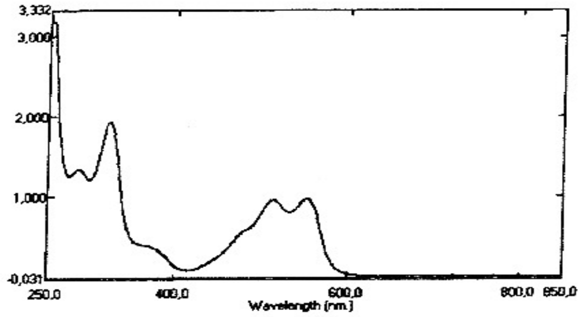

***Figure 5**. UV- visible spectrum of the DMPD radical cation ($DMPD^{\cdot +}$)*

The antioxidative efficiency was expressed in TEAC (TROLOX equivalent antioxidant activity) according to method, using the calibration curve plotted with different amounts of TROLOX (see Figure 6 for white wines and Figure 7 for red wines). The standard deviation is very low and the dose-response curve is highly reproducible. Inhibition of the absorbance at 505 nm is linear between 0.2 and 11 µg of TROLOX. The relation ship calculated within this range for the standard compound is:

A_{505} (inhibition) = 5.3 (µg of TROLOX) + 7.0 ;

r^2 = 0.987

It is observed that the red wines have a higher antioxidative activity (between 5.80% -10,2%) than the white wines. The white wines hane an antiradicalic efficiency lower than 3% (1,9% - 3,10%). The difference of antioxidative activity is explained on the basis of the differnt contain of antioxidative compounds.

***Tabel 5.**- Antioxidant Activity of tested wines*

Samples	Type of wine	Amount of polyphenols (ppm tanic acid)	Antioxidant activity (µg trolox)	Inhibition effect (%) $(1-A_f/A_0)x100$
		White wines		
S_1	C.*	288	1.8	14. 2
S_2	$M._1$.*	240	1.84	14. 9
S_3	$M._2$	378	1.9	15. 21
S_4	S_1	312	2.05	15. 50
S_5	S_2	332	2.1	15. 72
S_6	R_1	255	2.1	17. 08
S_7	R_2	281	2.2	17. 45
S_8	R_3	445	3.1	25. 7
		Red wines		
S_1	$C.S_1$	1780	5,8	38,0
S_2	C. S. $_2$	1400	6.0	38,6
S_3	M_1*	1230	6.12	39,0
S_4	M_2	1445	6.12	38,2
S_5	B.	1920	6.9	43,6
S_6	P. $N._1$	1675	6.9	44,2
S_7	P. N_2	1245	7.97	45,3
S_8	B.*	935	8.0	51,2
S_9	C.	1025	10.2	58,7

*On the label is mentioned "it contains sulfites"

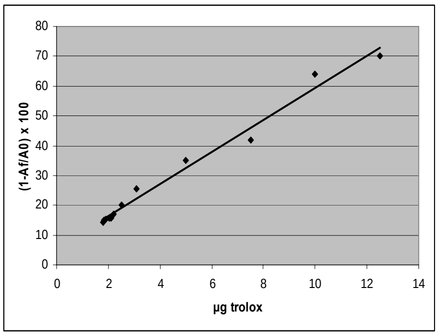

Figure 6. *Antioxidant activity for white wines*

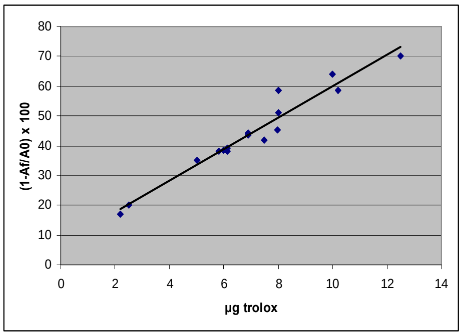

Figure 7. *Antioxidant activity for red wines*

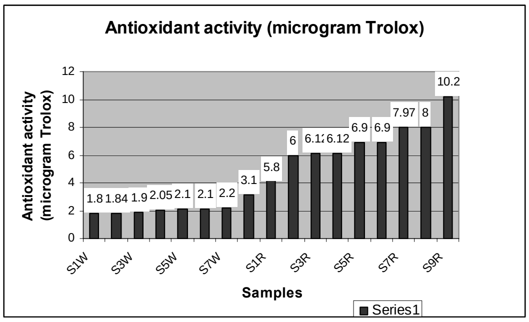

Figure 8. *Antioxidant activity of tested wines*

There is a correlation between the content of phenols and the TEAC of each red wine and a clear difference between the value of TEAC of red wine samples and the white ones. The total phenol content of the white wines is too low to account for their TEAC values (see Figure 8). This finding could be related to the addition of antioxidants such as sulfur dioxide, which are widely used as preservatives, in white wines.

Conclusions

In this paper a method to measure antioxidant power based on the DMPD colored radical cation is reported. The assay is particularly suitable for a largescale screening of white and red wines.

Studying the values of polyphenols and the the sulf values, there are some samples in which the polyphenols are in the highest concentration, although the „active sulf" is found less-sb unitary values or a little after 1. This observation allows us to accept that there are found some sorts of the wines of higher quality than others.

The contain of phenols and of „active SO_2" shows the antioxidative action of the analysed wine samples.

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References

Murphy, G.P., *Am. Canc. Soc.* 2, 23–45 (1997).

Yoshikawa, T., Naito,Y., Kondo, M., Food and diseases 2. In: *Free Radicals and Diseases*, Eds. New York: Plenum Press, 1997, pp 11–19.

Halliwell, B., Gutteridge, J.M.C., *Free Radicals in Biology and Medicine* UK: Oxford University Press, 1999, pp 60–67.

Buyukokuroglu M.E., Gulcin, I., Oktay, M., Kufrevioglu, O.I., *Pharmacol Res* 44:: 491– 494 (2001).

Tinttunen, S., Pekka L., Eur. Food Res. Techol, 201, 390-394 (2001).

Lopez – Velez, M., Martinez-Martinez, F., Del Valle – Ribes, C., *Crit. Rev. Food Sci. Nutr.*, 43(3), 233-238 (2003).

Pellegrini, N., Simonetti, P., Gardana, C., Brenna, O., Brighenti, F., Pietta, F., *J. Agric. Food Chem.*, 48(3),732-735 (2000).

Gheorghiță M., Muntean, C., Băduc, C.C., *Oenologie*, Ed. Sitech, Craiova, 2002.

Sauciuc, J., Țîbîrnă, C.R., Odăgeriu, G.H., Cotea, V.V., Patraş, A., „*Cercetări agronomice în Moldova*”,3 (4), 104-108 (1995).

Meir, S., Kanner, J., Akiri, B., Hadas, S.P., *J. Agric. Food Chem.* 43, 1813–1819 (1995).

Vincenzo, F., Verde, V., Giacomino, R., Ritieni, A., *J. Agric. Food Chem*., **47** (3), 1035-1039, 1999.

Țîrdea, C., Țîrdea, A., Sârbu, Gh.,*Tratat de vinificație,* Ed. Ion Ionescu, Iaşi, 2000.

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