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RESPIRATORY AND VASCULAR EFFECTS OF SOME FRACTIONS ISOLATED FROM RED WINE

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ABSTRACT - *We have isolated a series of fractions (T_1–T_6) from a sample of red wine and studied their influence on cellular respiration and behaviour of blood vessels, by in vitro analysis of striated muscle and liver of frog (Rana ridibunda, Pall), and of fragments of Wistar rat aorta artery. Different biological effects were registered, according to the type of tissues, composition of fractions (polyphenol proportion), and duration of registrations. The dominant effect on cellular respiration consisted in its stimulation, registered at fractions richer in polyphenols – T_2 and T_3, more pronounced than that produced by wine and more prominent at liver than at muscles. The same fractions clearly influenced the smooth muscles of aorta artery, determining an important vasodilatation effect. The other fractions had a weaker or inhibiting action on cellular respiration and did not show evident vascular effects. The results pointed out a series of useful pharmacological properties of certain studied wine fractions.*

Key words: fractions from red wine, physical-chemical parameters, respiratory effects, vascular effects

REZUMAT – Efecte respiratorii şi vasculare ale unor fracţii izolate din vin roşu*. S-a izolat o serie de fracţii (T_1–T_6) dintr-o probă de vin roşu şi s-a studiat influenţa lor asupra respiraţiei celulare şi a comportării vaselor sagvuine, lucrându-se in vitro pe muşchi striat şi pe ficat de broască (Rana ridibunda, Pall), precum şi pe fragmente de arteră aortă de şobolan Wistar. S-au înregistrat efecte biologice diferite, în funcţie de tipul ţesuturilor, compoziţia fracţiilor (proporţia de polifenoli) şi durata înregistrărilor. Efectul dominant asupra respiraţei celulare a constat într-o stimulare a acesteia, înregistrată la fracţiile mai bogate în polifenoli – T_2 şi T_3, mai pronunţată decât cea produsă de vin şi mai accentuată la ficat decât la muşchi. Aceleaşi fracţii au influenţat, în mod evident, musculatura netedă a arterei aorte, determinând un efect*

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vasodilatator important. Celelalte fracţii au avut o acţiune mai slabă sau inhibitoare asupra respiraţiei celulare şi nu au manifestat efecte vasculare evidente. Rezultatele au indicat o serie de proprietăţi farmacologice utile ale unora dintre fracţiile de vin studiate.

Cuvinte cheie: fracţii din vin roşu, parametri fizico-chimici, efecte respiratorii, efecte vasculare

INTRODUCTION

Different investigations pointed out the positive biological effects of some bioactive compounds from wine, especially of polyphenols present at higher rates in red wines (Burda and Olesznek, 2001; Cotea, 1985; Dell'Agli et al., 2004; Ursini and Sevanian, 2002). The literature shows that these compounds have antioxidant, anti-inflammatory, anti-atherogene, vasoactive, hepatoprotecting and redox modulator properties (Dell'Agli et al., 2004; Halliwell, 1993; Loeper et al., 1984; Neacşu et al., 2006, 2007).

Considering these aspects, this paper presents the results regarding the isolation of a series of fractions from a sample of red wine, as well as their effects on blood vessels and cellular respiration.

MATERIALS AND METHODS

The aim of this study was to analyse certain bioactive properties of phenolic compounds, which are present in a series of fractions isolated from wine. We have used a red wine having the following characteristics of composition: total acidity 6.63 g/L acetic acid; density at 20 ^{o}C, 0.9929 g/cm^3; alcoholic degree = 11.4 % v/v; residual sugars = 3.02 g/L; E.N (nonreducing matter) = 17.9 g/L; E.S.T. (Total dry matter) = 20.9 g/L. The wine had a relatively significant phenolic composition (D_{280} – total phenolic index) = 59.73, IFC (Folin Ciocâlteu Index) = 47.46 g/L gallic acid and IMn (permanganate index) = 39.28), being a coupage of Cabernet Sauvignon, offered for testing by "Vinia" Iaşi Company.

For extraction, we have used activated coal, prepared according to the following conditioning programme: a mixture of 200 mL methanol and 4 mL HCl fumans, with drying at 60 ^{o}C → 100 ^{o}C → 200 ^{o}C, for one day.

The codes of fractions extracted from activated coal are the following:

- T_1 – effluent from filtration of 200 mL wine, which we have treated firstly, interfered with 9 g activated coal;
- T_2 –washing of active coal with 50 mL acetone;
- T_3 –washing of active coal with 50 mL acetone;
- T_4 –washing of active coal with a mixture 25 mL acetone + 25 mL ethanol;
- T_5 –washing of active coal with a mixture 25 mL acetone + 25 mL ethanol;
- T_6 –washing of active coal with 50 mL methanol.

In a series of experiments, we have investigated *in vitro* cellular respiration, on fragments of striated muscle and liver from 5-8 living frogs (*Rana ridibunda*, Pall). Different groups of tissues were incubated in Warburg respiration vessels, isolated from

air, and containing physiological solution prepared with phosphate buffer M/15 (pH=7.4): control groups of muscles or liver, incubated in physiological solution without studied agents and groups treated by incubation in physiological solution containing red wine (sample P_0) or one fraction isolated from it (T_1, T_2, T_3 or T_6), at a concentration of 2 mL/100 mL.

The oxygen consumption by living cells was determined through the micromanometrical method (Nuță and Buşneag, 1977), at constant volume and temperature (20 ^oC), during 60 minutes. Reading of manometrical values and calculation of oxygen consumption (mm^3 O_2/g fresh tissue) were done every 15 minutes.

In another series of experiments, we have studied the vascular effects of wine fractions, by *in vitro* analysis of fragments (rings of 3–4 mm) from the descending aorta in Wistar white rats, in two variants: fragments of aorta with intact endothelium or with removed endothelium. Aorta rings were fixed, slightly tensioned, in organ baths, at a force of 100 mN and incubated at 37 ^oC, in 4 mL of Krebs-Hanseleit physiological solution, bubbled with carbogenes (95 % O_2 and 5 % CO_2), for balancing. Wine fractions were added in physiological solution, achieving a concentration of 2.5 mL/100 mL.

For registering the contractions of smooth vascular muscles, we have used force isometric transducers, coupled to a data acquisition system, with tracing a time-force contraction curve (seconds - mN).

The vascular effects of T_1–T_6 fractions have been studied in two different experimental situations: on aorta fragments with relaxed musculature (under base conditions), for testing the reactivity of relaxed muscle, by observing the contractile effect *per se* and on pre-contracted fragments by treatment with phenylephrine 10^{-7}–10^{-6} M and K^+ 40–70 mM. This was done for observing the vasodilator effect of studied fractions, on already contracted smooth vascular muscle, as compared with the effect of carbachol (10^{-6} M) as reference agent releasing nitric oxide (NO), which is an endothelial and vasodilator factor (Hăulică, 2007; Karp, 1996).

On pre-contracted aorta fragments, wine fractions were used at the plateau phase of contraction, determined by phenylephrine.

The obtained data were statistically calculated according to the Student test; the values of treated groups being compared with the ones of control groups.

RESULTS AND DISCUSSION

From previous experiments, we have noticed that, by using PVPP (polyvinylpolypyrrolidone) and/or activated coal, we achieved an efficient separation of the mixture of phenolic compounds. PVPP could not be used because of its relatively low adsorption capacity, while active coal cannot be placed in a chromatographic column, because it is strongly compacted or, if used in sinterized state, it loses quickly its properties. In this case, the adsorption-desorption technique has required a modification of the method, being achieved a vacuum aspiration on a larger area, in comparison with conventional techniques. In the table below there are presented the values of dielectrical constants of some solvents used for the elution of phenolic compounds:

Substance	ε Relative dielectrical constant
acetone	20.7
ethanol	24.3
methanol	32.63
water	78.54

From a series of experiments, we could notice that a differentiated extraction and dilution of phenolic compounds were efficient at substances with ε comprised between 20 and 32, such as acetone, methyl-ethyl-cetone and superior alcohols. Anthocyanic compounds do not prefer certain solvents, but, as already known, in this case, too, their solubilization degree in water is low (Cotea and Sauciuc, 1988). From this study, we removed some substances that, although with a good efficiency in extraction, were not used because of their high toxicity degree (acetonitrile, benzyl chloride, cyclohexyl methyl ketone, etc.)

Superposing spectra from fractions T_1 to T_6 on diverse interest fields is presented in *Figure 1*.

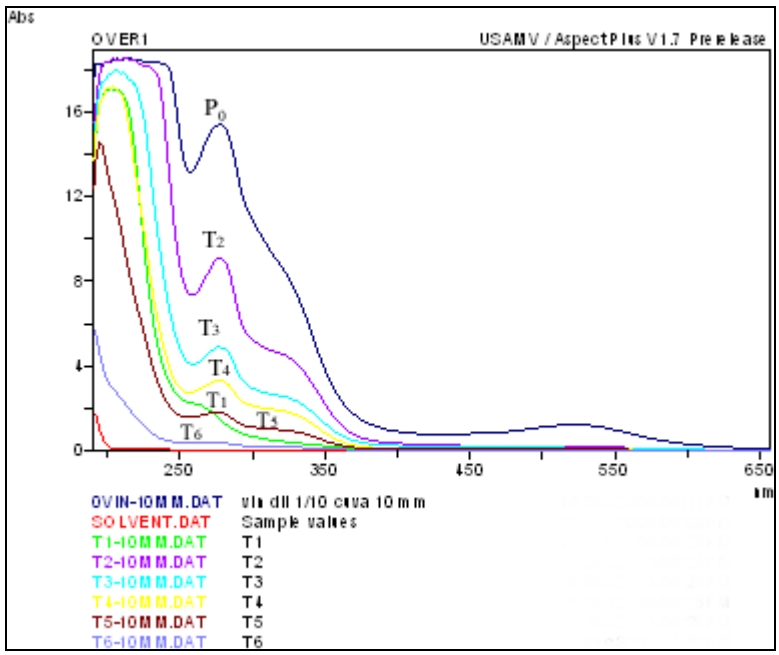

Fig. 1 - UV-VIS spectrum of eluted fractions from activated coal

After separation, fractions were analysed spectrally. Results were presented in the following table:

Fraction	D_{280}	IFC g/L gallic acid	Quantity of anthocyans mg/L
T_0	59.73	47.46	624.82
T_1	19.47	16.19	175.75
T_2	45.27	39.74	403.07
T_3	30.72	27.27	232.89
T_4	22.43	22.73	192.20
T_5	20.72	20.49	190.65
T_6	20.06	11.27	34.32

As we can see, there was a clear separation of phenolic compounds according to the dielectrical constant of elution solvent. Thus, anthocyans and perhaps, proanthocyans were extracted in T_2 and T_3 fractions, where they found again quantitatively as values, but because the used method – of pH variation at 520 nm –was not typical only of anthocyans, it was possible that only one part of proanthocyans appeared at determination subsequently to the extraction, hydrolysing in acid medium. Spectrally, as well as from D_{280} (total polyphenolic index) values and IFC (Folin-Ciocâlteu Index), we noticed that in T_4, T_5 and T_6 fractions there were no anthocyans, but tannins and adducts of phenolic compounds showing low biological activity.

The obtained data pointed out some important aspects of the biological effects in studied wine fractions.

As concerns cellular respiration, we found out that the intensity of oxygen consumption by cells was differently influenced by wine fractions, according to the nature of studied tissue and fractions, as well as to the duration of registrations. Therefore, we found a progressive increase in oxygen consumption of determinations, during 60 minutes, at all tissue groups; however, the growth rate has gradually decreased with time, because of the diminution in cell energetic substratum from isolated tissues and of the oxygen from closed testing vessel, and due to the accumulation of cellular metabolic residues (Lehninger, 1987; Karp, 1996; Hăulică, 2007).

The reactivity of muscular and hepatic tissues to the applied treatments are different, correlated to structural-functional characteristic features of cells from tissues, because striated and smooth muscular cells are excitable-type ones, while the hepatic cells are non-excitable, having especially a metabolic role (Hăulică, 2007).

In untreated control groups, we found that the intensity of aerobe cellular respiration was initially different in the two tissues. After 15 minutes, the liver values were lower (89.45 %), as compared with the muscle ones, but after 60 minutes, the oxygen consumption was similar at both tissues (99.83 % at liver, compared to the muscle).

The treatment applied has required a different response of the two tissues (*Figures 2 and 3*). Therefore, wine (P_0) determined a stimulation of the cellular

respiration, as compared with the control, both at muscles (50.99 % at 60 min), and at liver (13.72 % at 60 min). The values registered at liver were lower as compared to the muscles ones (75.18 % every 60 min).

The studied wine fractions have shown specific respiratory effects according to their nature and the tissues on which they acted (*Figures 2 and 3*). Thus, at muscles (*Figure 2*), fractions T_1, T_2 and T_6 had a more diverse action than at liver (*Figure 3*), being found, however, a diminution effect of cellular respiration. In case of fractions T_1, T_3 and T_6, the inhibiting effect was higher in the first 15 minutes (24.91 % at T_1 and 32.35 % at T_3), and then more reduced, until 4.23 % after 60 minutes, at T_3; the T_1 fraction has shown even a stimulating effect after 60 minutes, when oxygen consumption was higher by 8.10 %, compared to the control.

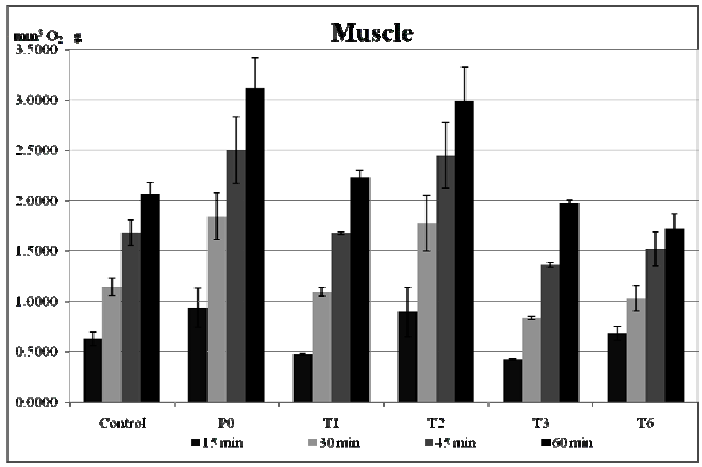

Fig. 2 – Effect of fractions on cellular respiration at muscles

A special aspect was noticed at the T_2 fraction, which determined a stimulation of muscular cell respiration on the entire period of registrations; after 60 minutes, the values were by 30.46% higher than in the control group. Unlike muscle, at liver, all the fractions have induced a stimulation of aerobe–cellular respiration (*Figure 3*). The effect was quite strong and stable on the entire period of registrations; after 60 minutes, the values were higher, as compared with the ones from muscles, by 116.67% at the T_1 fraction, 110.94% at T_2, 156.19% at T_3 and by 124.23% at T_6.

The T_2 fraction also had a special effect on liver, determining the highest stimulation of cellular respiration, as compared with the other fractions; the registered values were higher by 132.96%, after 15 minutes and by 44.98%, after 60 minutes, compared to the control (*Figure 3*).

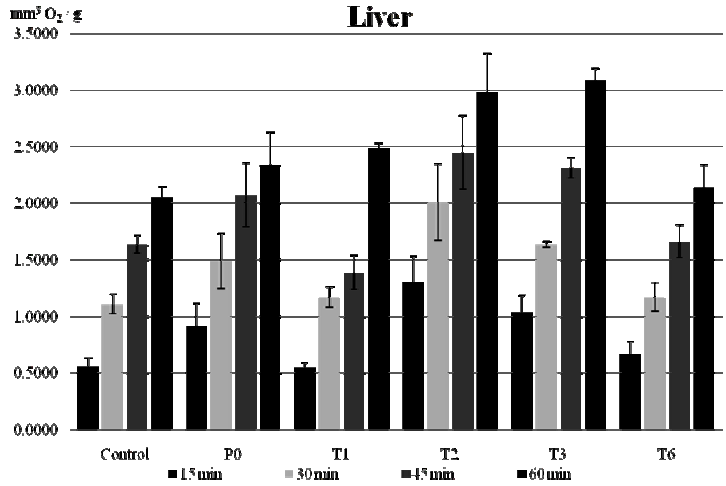

Fig. 3 – Effect of fractions on cellular respiration at liver

The specific aspects of studied fractions on cellular respiration at the two tissues derived both from physical-chemical characteristics of these fractions and from the different reactivity of the two cell types. Thus, T_1 and T_6 fractions have a relatively similar composition (*Figure 1*), so, their effects are also close. However, in T_6 there are some traces of methanol with toxic properties (Cotrău et al., 1991), inducing a tendency of respiration inhibition, especially at muscle (*Figure 2*). In addition, liver has the capacity of reducing toxicity (Cotrău et al., 1991; Hăulică, 2007), which diminishes the inhibiting effect of this fraction at liver, as compared with muscular tissue.

T_2 and T_3 fractions were obtained by a similar method, but their respiratory effects were different (T_2 – stimulation at both tissues, T_3 – weaker stimulation at liver and inhibition at muscle), probably because of concentration differences of some constituents, since T_2 has resulted from the first stage of active coal washing with acetone, and T_3, from the next stage. The effects of intensifying oxygen cellular consumption point out a stimulation of aerobe cellular respiration processes within Krebs cycle, correlated to an intensification of oxidative phosphorylation, which determine a superior energetic level of treated cells (Karp, 1996; Lehninger, 1987). These effects exhibit the antioxidant, anti-inflammatory, vasodilator, hepatoprotecting and redox modulator properties of polyphenols (Dell'Agli et al., 2004; Halliwell, 1993; Loeper et al, 1984; Neacşu et al., 2007) from wine composition and studied fractions. However, we noticed that wine had more stable effects, and among the studied fractions, T_2 had a more pronounced stimulating action at the level of both tissues.

The vascular effects of fractions from wine also pointed out some important specific aspects. Thus, acting on normal aortic fragments, we found out that none of fractions $T_1 - T_6$, applied at base state, had vasoconstriction effects *per se* on smooth vascular musculature. The fractions applied in the contraction plateau had different effects, according to their nature, on pre-contracted aortic preparations with phenylephrine. Thus, the fraction T_1 did not show any effect. Nevertheless, the T_2 fraction has determined a significant vasodilator effect of 35±7 % of the contraction caused by phenylephrine (*Figure 4*), which could have positive consequences on blood circulation.

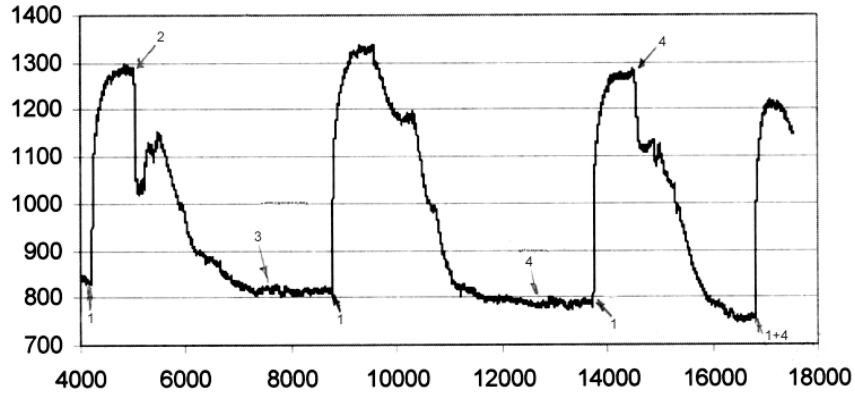

Fig. 4 – Vasodilator effect of applying 100 μL T_2 in pre-contraction of phenylephrine 10^{-5} M on endothelized preparation: 1-phenylephrine, 2-carbachol, 3-T_1 (1/1), 4-T_2 (1/1)

This effect was seen only at the aortic preparations with intact endothelium and not at the desendothelized ones (*Figure 5*). This fact points out the role of endothelium in the relaxation of smooth muscle of the vascular wall and draws the attention on negative effects of its deterioration in atherosclerosis. These effects can be diminished by the protection and antioxidant action of polyphenols from wine (such as T_2).

The vasodilator effect of T_2 fraction was strong enough, allowing the drawing of a rate-effect curve, according to successive dilutions, from this fraction. Actually, T_2 had the highest stimulating effect on cellular respiration (*Figures 2 and 3*). The T_3 fraction also had a vasodilator effect at preparations with intact endothelium, but more reduced than T_2, only 26±7% of the contraction induced by phenylephrine. Drawing a rate-effect curve was not possible, because it needed higher concentrations of active substance. Fractions T_4, T_5 and T_6 had no clear effects on smooth arterial musculature, neither under base conditions, nor at the application in the contraction plateau determined by phenylephrine.

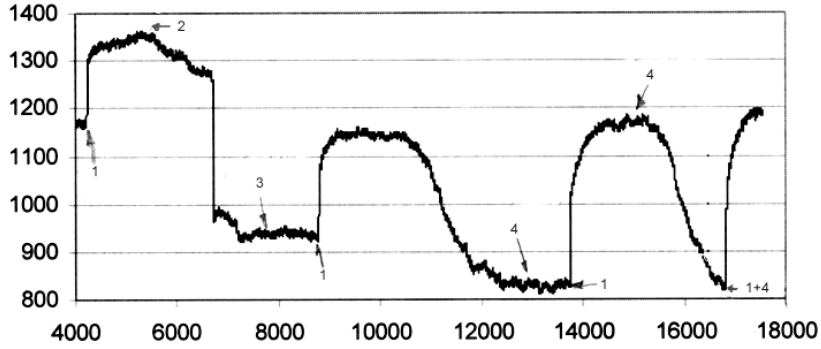

Fig. 5 – Vasodilator effect of applying 100 µL T_2 in pre-contraction of phenylephrine 10^{-5} M on desendothelized preparation: 1-phenylephrine, 2-carbachol, 3-T_1 (1/1), 4-T_2 (1/1)

The vasodilator effects of T_2 and T_3 fractions are correlated to the presence of the highest quantity of phenolic compounds, as compared with the other fractions (*Figure 1*). The vasodilator action, shown by polyphenols from wine, was noticed in other studies, too. It could be explained by the property of polyphenols to interact with NO, as vasodilator endothelial factor and to determine the increase of the expression of endothelial NO-synthase (Dell'Agli et al., 2004).

The results obtained by us enrich the database concerning the biological effects of compounds from wine and studied fractions, pointing out their useful pharmacological properties. For explaining these phenomena, additional investigations are still necessary.

CONCLUSIONS

At the experiments having active coal as support and according to the way the elution was carried out, we have noticed significant biological effects at the ketonic fractions, weaker effects at the ethanolic ones and similar effects at watery and methanolic solutions.

The dynamics of tissue respiration is different from two viewpoints: type of respiratory tissue and nature of analysed fractions; it also depends on the duration of determinations. At the hepatic tissue, the action of phenolic compounds is different from the one of muscular tissue, because hepatic cells are not of excitable-type, like the muscular ones, and have different metabolic specific features. The respiratory effects of studied fractions are, generally, stronger and more stable at the hepatic cells.

As concerns the vasodilator and antioxidant capacity, there are clear differentiations at the experimental variants, according to the nature of studied

fractions and the presence of vascular endothelium. Thus, the anthocyan rich fractions (T_2 and T_3) are efficient as vasodilator agents, unlike the others, which have not vascular activity, the vasodilator effects being revealed only at vascular fragments with intact endothelium and not at desendothelised ones.

The stability of phenolic compounds is low in watery mediums at the physiological pH (~7.4), at which experiments were carried out; the stabilization of these compounds appears from a 2.5 pH towards a more acid medium; one must take into account this fact when interpreting the experimental data.

By using usual materials for technological operations, one may achieve a rough separation of phenolic acids from anthocyans, but a separation until the level of molecules can be done only by the preparative and further analytical HPLC technique, which allows the separation in continuous solvent gradient of molecules, on each of the materials used in this study.

REFERENCES

Burda S., Oleszek W., 2001 – *Antioxidant and antiradical activities of flavonoids*. J. Agric. Food Chem., 49, 2774-2779

Cotea V.D., 1985 – *Tratat de Oenologie, vol. I*, Ed. Ceres, Bucureşti, 224-250

Cotea V.D., Sauciuc J., 1988 – *Tratat de Oenologie, vol. II*, Ed. Ceres, Bucureşti

Cotrău M., Popa Lidia, Stan T., Preda N., Kincses-Ajtay Maria, 1991 – *Toxicologie*, Ed. Didactică şi Pedagogică, Bucureşti, 119-120

Dell'Agli M., Buscialà A., Bosisio E, **2004 –** *Vascular effects of wine polyphenols*. Cardiovascular Research, 63, (4), 593-602

Halliwell B., 1993 – *Antioxidant in wine,* Lancet, 341, 1538

Hăulică I., 2007 – *Fiziologie umană*, Ediția a III-a, Ed. Medicală, Bucureşti

Karp G., 1996 – *Cellular and Molecular Biology*, John Willey and Sons Inc., New York, Chichester, Brisbane, Toronto, Singapore, 117-205; 675

Lehninger A.L., 1987 – *Biochimie*, vol. II. Ed. Tehnică, Bucureşti, 473-503

Loeper J., Goy J., Emerit J., 1984 – *Lipid oxidation in human and experimental atherosclrosis*. Bull. Acad. Natl. Med., 168 (1-2), 91-97

Neacşu I., Cotea Irina Anca, Niculaua M., Cotea V.V., 2007 – *Hepatoprotecting effects of some bioactive principles from red wins*. Electronic version, XXXth OIV World Congress of Vine and Wine, Budapest, 10-16 june 2007, Section 4: Safety and Health – Wine in health and reseach, wine and nutrition, wine in pathology prevention.

Neacşu I., Rotinberg P., Gherghel D., Mihai C., Cotea V.V., Zănoagă C. V., Niculaua M., 2006 – *Influence of natural polyphenolic fractions on cellular respiration*. Cerecetări Agronomice Moldova, XXXIX, 4(128), 33-40

Nuță G., Buşneag C., 1977 – *Investigații biochimice*, Ed. Didactică şi Pedagogică, Bucureşti, 283-286

Ursini F., Sevanian A., 2002 – *Wine, polyphenols and optimal nutrition*. Annals N.Y. Acad. Sci., 957, 202-209