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INFLUENCE OF SOME FRACTIONS ISOLATED FROM RED WINE ON OXYGEN CELLULAR CONSUMPTION

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ABSTRACT – In this study, we carried out the analyse of some polyphenolic fractions obtained from a sample of red wine by solid-liquid repartition. The wine selected sample was obtained by mixing noble wine varieties, with the purpose of realising a great concentration of phenolic compounds. The fractions obtained from this special sample were firstly chemically analysed (total quantity of polyphenols, total quantity of anthocyans, Folin-Ciocâlteu index and permanganate index), and then were correlated with their specific effects on the intensity of oxygen respiratory consumption of the batracian muscle and hepatic cells, carried out by the Warburg micromanometrical method. Different biological effects (values of oxygen cellular consumption) were recorded, according to the type of cells, composition of fractions (polyphenol quantity) and duration of records. The obtained results evidenced a specific influence of the studied fractions on the respiratory and energetic cellular processes, indicating a series of useful pharmacological properties of some wine fractions.

Key words: red wine fractions, chemical and bioactive properties, respiratory effects

REZUMAT - Influenţa unor fracţii izolate din vinul roşu asupra consumului celular de oxigen. Studiul a realizat analiza unor fracţii polifenolice extrase prin repartiţie solid-lichid dintr-un eşantion de vin roşu, superior calitativ. Acesta a fost preparat prin cupajarea unor soiuri nobile superioare, cu scopul de a avea o concentraţie cât mai mare de compuşi polifenolici. La fracţiile obţinute s-au urmărit proprietăţile chimice ale componenţilor (cantitatea de polifenoli totală, cantitatea de antociani totală, indicele Folin-Ciocâlteu, indicele de permanganat), în corelaţie cu efectele lor specifice asupra intensităţii consumului respirator

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de oxigen al celulelor musculare şi hepatice de batracieni, evidenţiat prin metoda micromanometrică Warburg. S-au înregistrat efecte biologice diferite ale intensităţii consumului celular de oxigen, în funcţie de tipul ţesuturilor (celulelor), compoziţia fracţiilor (cantitatea de polifenoli) şi durata înregistrărilor. Rezultatele au evidenţiat influenţa specifică a fracţiilor studiate asupra proceselor respiratorii şi energetice celulare, indicând o serie de proprietăţi farmacologice utile ale unora dintre aceste fracţii.

Cuvinte cheie: fracţii din vin roşu, proprietăţi chimice şi bioactive, efecte respiratorii

INTRODUCTION

Numerous studies have emphasized the positive effects of some bioactive compounds from wine, especially of the polyphenols, which are found at higher rates in red wines (Burda and Olesznek, 2001; Cotea, 1985; Cotea and Sauciuc, 1988; Dell'Agli et al., 2004; Ursini and Sevanian, 2002; Watkins, 1977; Neacşu et al., 2007, 2008). Thus, they showed that these bioactive compounds had antioxidant, anti-atherogenic, vasodilatatory, anti-inflammatory, membranotrope, hepatic protection and redox modulator properties, which are also mentioned for explaining the so-called "French paradox" concerning the positive effects of wine moderate consumption (Renaud and Lorgeril, 1992).

In a previous paper (Cotea et al, 2008), we have shown the aspects of the specific action of some fractions isolated from a sample of red wine on the cellular respiration processes and on the behaviour of testing animal blood vessels. Based on these results, we have followed in this paper the specific effects of other fractions, separated from the same wine sample, on the intensity of the oxygen respiratory consumption by batracian muscle and hepatic cells, correlated with the chemical properties of studied fractions.

MATERIALS AND METHODS

In this study, our goal was to emphasize the bioactive properties of some fractions, which are different from the previously studied ones (Cotea et al.,2008), with a various polyphenol content, investigating their specific effects on the respiratory and energetic cellular processes, according to their composition and their physico-chemical properties.

The polyphenolic fractions ($P_1 – P_8$) were extracted by solid-liquid repartition from a sample of high quality red wine, achieved by the coupage of noble wines, for obtaining an increased concentration of polyphenolic compounds. This wine had the following characteristics of composition: total acidity -6.63 g/L acetic acid; density of 0.9929 g/cm^3 at 20 ^oC; alcoholic degree - 11.4% v/V; residual sugars -3.02 g/L; nonreducing extract - 17.9 g/L; total dry mater- 20.9 g/L; D_{280} - 59.73; I_{FC} - 47.46 g/L gallic acid and I_{Mn} -39.28.

For each fraction, they established the values for D_{280} (total polyphenolic index), quantity of anthocyans and I_{FC} (Folin-Ciocâlteu index).

The intensity of cellular respiration was studied *in vitro*, on homogenized fragments of striated muscle and liver from five living frogs (*Rana ridibunda*, Pall) for each experimental variant, the oxygen cellular consumption being determined by the Warburg micromanometrical method (Nuță and Buşneag, 1977). Reading of manometrical values and calculation of the oxygen consumption (mm^3 O_2 / g fresh tissue) were done at intervals of 15 minutes, during one hour.

The work was realised on various batches of tissues incubated in Warburg respiration vessels, with different treatments: muscle and liver control batches, incubated in the normal Ringer physiological solution (NR), without fractions and NR treated batches, which contained 2 mL/100 mL of the one of studied fractions or of the red wine from which we isolated them (P_0 sample).

The obtained data were statistically processed according to the Student test, the values of treated batches being reported as percentage to the control ones.

RESULTS AND DISCUSSION

Based on the obtained results, we evidenced some typical aspects of the effects of studied wine fractions on the intensity of cellular respiration processes.

Thus, it was noticed that the intensity of oxygen respiratory consumption was differently influenced by wine fractions, according to their chemical characteristics, nature of studied cells and duration of records (*Table 1*). Therefore, a progressive increase in the values of oxygen consumption was found during one hour, in all the tissue groups, irrespective of the applied treatment.

The growth rate has gradually decreased along with the increase in the experiment duration, due to the diminution of the energetic substratum from isolated cells and of the oxygen consumption from closed experience vessels and to the accumulation of metabolic residues in the tested cells (Haulică, 2007; Karp, 1996; Lehninger, 1987).

The two types of tested cells have different structural-functional characteristics – the muscular ones being of excitable type and the hepatic ones, of non-excitable type, with metabolic role (Haulică, 2007; Karp, 1996), this aspect determining their different response to the action of studied wine fractions. Thus, after 60 minutes, at the untreated control, the values of oxygen consumption were generally lower in the hepatic cells than in the muscular ones (*Table 1*). At the groups treated with wine or different fractions from wine, the sense and amplitude of the response depended on the cell type, but the compositional characteristics of fractions had a greater influence. So, after 60 minutes of experiment, we noticed that the wine treatment (P_0) has resulted in intensifying the cellular respiration in both tissues, compared with the control (100%), but, at the same time, the stimulation of the respiratory process was greater in muscle (150.99%) compared to that of liver (113.72%).

Table 1 - Respiratory effect of wine fractions

Sample	Indices	Tissue/time/oxygen consumption mm^3 O_2 / g							
		Muscle				Liver			
		15	30	45	60	15	30	45	60
Control	$\bar{x}$	0.6266	1.1467	1.6807	2.0632	0.5604	1.1107	1.6384	2.0597
	SE	0.0667	0.0869	0.1275	0.1167	0.0715	0.0872	0.0782	0.0833
	VC %	30.11	21.44	21.46	16	36.09	22.21	13.5	11.45
	% C	100	100	100	100	100	100	100	100
P_0 wine	$\bar{x}$	0.9339	1.8437	2.4978	3.1153	0.9203	1.4899	2.0734	2.3422
	SE	0.1939	0.2328	0.3301	0.3053	0.1906	0.2438	0.2822	0.2869
	VC %	30.86	30.93	32.37	24.01	30.72	40.08	33.33	30.01
	% C	149.04	160.8	148.62	150.99	164.22	134.14	126.55	113.72
P_1	$\bar{x}$	1.2034	2.0551	2.6761	3.5984	0.7095	1.3318	1.0774	2.5124
	SE	0.2276	0.3278	0.253	0.2092	0.1101	0.2428	0.3171	0.4318
	VC %	43.48	45.11	26.75	16.44	43.88	41.57	42.53	48.61
	% C	192.05	179.22	159.22	174.71	126.06	119.91	104.21	121.98
P_2	$\bar{x}$	0.5829	1.3304	1.2865	2.2938	0.6713	1.1651	1.8804	2.4623
	SE	0.0083	0.0908	0.0115	0.0468	0.0302	0.0869	0.1029	0.1311
	VC %	3.19	15.26	1.44	4.56	10.07	16.67	12.23	11.91
	% C	93.03	116.02	106.29	119.18	119.79	104.9	114.77	119.55
P_3	$\bar{x}$	0.6663	1.4086	1.94	2.1523	0.6513	1.349	1.8467	2.3337
	SE	0.0936	0.07	0.0794	0.0468	0.0526	0.0322	0.0474	0.1151
	VC %	31.44	11.11	9.15	4.86	18.06	5.34	5.74	11.03
	% C	106.33	122.84	115.43	104.32	116.22	121.45	112.71	113.3
P_4	$\bar{x}$	0.5479	1.2825	1.531	1.8382	0.3773	1.0035	1.3528	1.5681
	SE	0.109	0.1169	0.1189	0.1435	0.0356	0.0854	0.1736	1.586
	VC %	44.48	20.38	17.36	17.46	21.08	19.04	28.69	22.62
	% C	87.44	111.84	91.09	89.09	67.33	90.35	82.57	76.13
P_5	$\bar{x}$	0.4603	0.7422	0.9374	1.4109	0.6438	0.8616	1.426	1.8079
	SE	0.0518	0.0503	0.0303	0.1691	0.0218	0.1498	0.0953	0.541
	VC %	25.19	15.14	7.24	26.8	7.56	38.87	14.95	6.69
	% C	73.46	64.72	55.77	68.38	114.88	77.57	87.04	87.77
P_6	$\bar{x}$	0.6327	0.9769	1.178	1.6579	0.8162	1.3933	1.8409	2.3595
	SE	0.027	0.1215	0.1079	0.1079	0.0343	0.1217	0.0767	0.0872
	VC %	9.54	27.8	20.49	14.55	9.4	19.53	9.32	7.67
	% C	100.97	85.19	70.09	80.35	145.65	125.44	112.36	123.29
P_7	$\bar{x}$	0.791	1.4051	2.0044	2.4989	0.8945	1.5232	2.1418	2.7333
	SE	0.1345	0.1813	0.1565	0.1733	0.1302	0.1689	0.2364	0.2404
	VC %	38.02	28.85	17.46	15.51	32.54	24.79	24.68	19.66
	% C	126.24	122.53	119.26	121.12	159.62	137.14	130.72	132.7
P_8	$\bar{x}$	0.7722	1.2141	1.7066	2.0932	0.7059	1.4233	2.0947	2.6924
	SE	0.1993	0.1921	0.2351	0.1659	0.1943	0.1628	0.1912	0.2427
	VC %	37.44	34.38	30.81	17.72	41.54	25.58	20.41	20.15
	% C	123.24	105.88	101.54	101.45	125.96	128.14	127.85	130.72

($\bar{x}$ - mean value, SE – standard error, VC – variability coefficient, %C – % from control batch)

The respiratory effects of the studied fractions were various, due to the different composition characteristics, besides the specific reactivity of the two cell types. Thus, the P_1, P_2 and P_3 fractions had a stimulating effect on oxygen consumption in both tissues, but the most intense action was determined by the P_1 fraction, especially in muscular cells (after 60 minutes, 174.41% in muscle and 121.98 % in liver), compared with the control, because of a higher polyphenols content (*Table 1*).

The P_6 fraction had an inhibiting effect on muscular respiration (80.35 %) and a stimulating one on hepatic respiration (123.29 %). The P_4 and P_5 fractions inhibited the cellular respiration both in muscles (P_4 – 8909 %, P_5 – 68.38 %) and in liver (P_4 – 76.13 %, P_5 – 87.77 %), this effect being, probably, due to a different composition of these fractions.

The intensification of oxygen cellular consumption by some fractions implies a stimulation of the cellular aerobe respiration processes and of the activity of involved enzymes within the Krebs cycle, in correlation with the intensification of oxidative phosphorylation; this results in increasing the ATP (adenosine triphosphate) synthesis and in a superior energetic balance of treated cells (Karp, 1996; Lehninger, 1987). These respiratory effects are correlated to antioxidant, anti-inflammatory, vasodilatatory, anti-atherogenic, and redox modulators properties of polyphenols from wine composition and studied fractions (Dell'Agli et al., 2004; Helliwell, 1993; Loeper et al., 1984, Neacşu et al., 2007).

The respiratory and energetic effects of the P_1 – P_6 fractions are similar to those of the T_1 – T_6 fractions, isolated from the same wine, which were studied in a previous work (Cotea et al., 2008). Generally, they are more homogenous as concerns the stimulation of aerobe cellular respiration in both cell types. The most intense and the most stable stimulating effects were found in the P_1 and P_3 fractions, as well as in the initial wine (P_0).

The P_7 and P_8 fractions are generally inducing a stimulation of respiratory processes in both cell types, but the effect of the P_7 fraction is much higher, especially in hepatic cells (132.7 % hepatic cells and 121.12 % muscular cells), while the effect of the P_8 fraction is practically found only in hepatic cells, being annulated after 15 minutes in the muscular cells.

CONCLUSIONS

The results of this study show some specific effects of the fractions isolated from red wine, expressed by the modulation in the intensity of oxygen respiratory consumption by muscular and liver cells, having different senses and amplitudes, according to cell type, composition of fractions (quantity of polyphenols) and duration of records.

Our results pointed out, especially, the effects of some fractions of stimulating the cellular respiratory and energetic processes, showing some of their useful pharmacological properties.

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