Antioxidant properties of the phenolic fraction of Sardinian wines

D. Loru[1]*, M. Deiana[1], A. Incani[1], A. Atzeri[1], A. Rosa[1], M.P. Melis[1], B. Cabboi[1], L. Hollecker[2], M.A. Dessì[1]

[1] Dipartimento di Biologia Sperimentale, Sezione di Patologia Sperimentale, Università degli Studi di Cagliari, Cittadella Universitaria SS 554, 09042 Monserrato (CA), Italy
[2] SARAS Ricerche e Tecnologie S.p.A., 5a strada ovest, trav. c, Z.I. Macchiareddu, 09032 Assemini (CA), Italy
* dloru@unica.it

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Abstract

The aim of this study was to compare the antioxidant capacities of the phenolic fraction of wines from* Vitis vinifera *grapes cultivated in Sardinia (three native: Cannonau, Malvasia, Vermentino and three non-native types: Cabernet-sauvignon, Chardonnay and Sauvignon), in simple* in vitro *systems. All the extracts showed a significant antioxidant activity, beeing the native wine extracts the most active in inhibiting the lipid peroxidation process.

Introduction

The antioxidant potential of wine, one of the key elements of the Mediterranean diet, is very high, due to the presence of phenolic compounds, whose many biochemical and pharmacological activities are related to their antioxidant properties [1]. Experimental evidence suggests that ingestion of antioxidant compounds inhibits oxidative stress associated diseases [2]. We evaluated the antioxidant effect against the lipid peroxidation process of the phenolic fraction of wines from grapes cultivated in Sardinia during linoleic acid and cholesterol autoxidation and in TBH treated intestinal Caco-2 cells.

Materials and methods

The wine extracts were prepared as methanolic extracts and analysed as previously described [3]. The main phenolic compounds quantified are shown in Tab. 1.
Linoleic acid (LN) autoxidation was conducted in dry state, at 37°C for 32 h, as previously described [4]. Cholesterol oxidation was conducted in dry state, at 140°C for 90min, as previously described [5]. Analyses of pure lipids and major oxidation products were carried out with a HPLC-DAD [4,5]. For experimental studies differentiated Caco-2 cells (from ECACC, Salisbury, Wiltshire UK), with enterocytic features,

Compounds	mg/kg of freeze-dried grapes					
	Cannonau	Cabernet	Malvasia	Vermentino	Chardonnay	Sauvignon
Hydroxybenzoic acids						
Gallic acid	59.30	80.74	61.61	1.34	0.76	0.78
Hydroxycinnamic acids						
trans-caftaric acid *	47.01	0.39	11.44	5.53	n.d.	n.d.
trans-coutaric acid *	81.43	1.86	11.63	1.86	0.40	0.86
trans-fertaric acid *	45.42	1.41	19.76	14.65	9.92	12.31
Stilbenes						
cis-resveratrol-3-O-glucoside**	2.78	0.55	0.97	0.09	0.06	0.07
Flavanols						
Catechin	9.97	11.19	0.21	0.02	0.30	0.04
Epicatechin	3.38	6.47	n.d.	n.d.	0.08	n.d.
Procyanidin B1	49.69	68.44	0.39	n.d.	1.15	n.d.
Procyanidin B2	11.50	23.58	n.d.	n.d.	0.16	n.d.
Flavonols						
Myricetin	5.30	4.77	0.03	n.d.	0.02	n.d.
Quercetin	15.07	6.43	0.44	0.12	0.12	0.05
Kaempferol	6.14	0.36	0.06	0.02	n.d.	n.d.
Myricetin-3-O-glucoside ***	9.00	0.65	n.d.	n.d.	n.d.	n.d.
Quercetin-3-O-glucuronide ***	17.39	4.03	0.06	0.01	n.d.	n.d.
Anthocyanins						
Malvidin_3_O_glucoside	13.34	20.59				
Malvidin_3_O_trans_p_coumarylglucoside	20.84	31.88				

n.d.: not determined.
(*) quantified as mg/kg equivalents of gallic acid
(**) quantified as mg/kg equivalents of procyanidin B1
(***) mg equivalenti di Quercetin-3-O-glucoside

Table 1. Quantitative analysis of major phenolic compounds in grape extracts

at passage 45-60, were plated at a density of about $1x10^5$/ml. Caco-2 cells were pretreated with the different concentrations of wine extracts (25-100µg/ml; 30min) and treated with TBH (2.5mM; 2h). The extent of oxidation was evaluated as malondialdehyde (MDA) formation, measured with the TBARS method [5]. Statistical significance was assessed by analysis of variance ANOVA, using the software Graph Pad INSTAT(n=9).

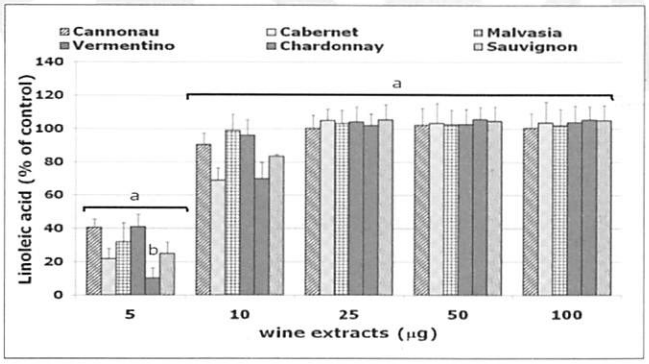

Figure 1. LN values measured during the autoxidation of LN at 37°C for 32h in the presence of wine extracts. a=p<0.001, b=p<0.01 vs. controls

Results

During the autoxidation of LN (Fig.1) wine extracts showed a significant inhibition of the oxidation process from the concentration of 5µg.

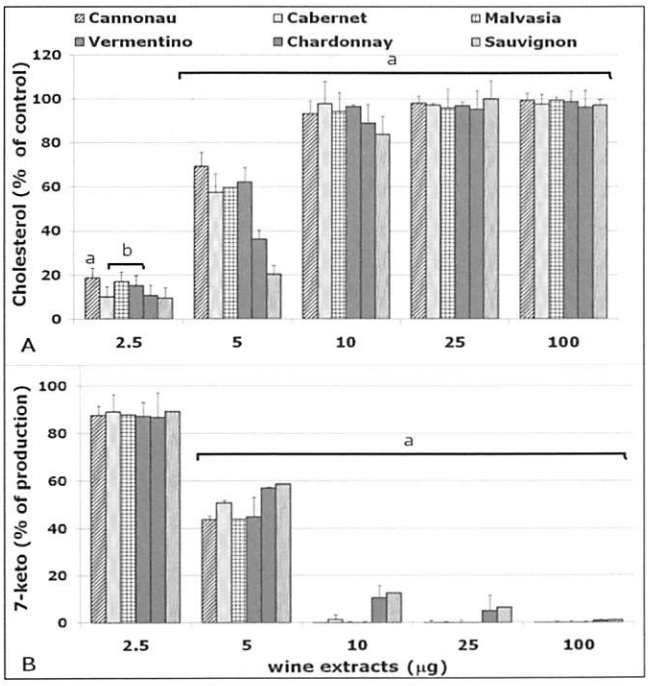

Figure 2. Cholesterol values (A) and 7-keto (B) measured during the autoxidation of cholesterol at 140°C for 90min in the presence of wine extracts

The extracts of native cultivars (Cannonau, Malvasia, Vermentino) exerted total protection from 10µg; the others from 25µg. The extracts of native cultivars were more effective also in preserving cholesterol from oxidative degradation, exerting a significant protection from 5µg (Fig.2A). All the extracts showed total protection from the concentration of 10µg and prevented the total formation of 7-ketocholesterol (7-keto) from a concentration of 10µg (Fig.2B). Caco-2 cells (Fig.3) pretreated with the extracts of native cultivars showed a significant reduction of MDA production from the concentration of 25µg/ml.

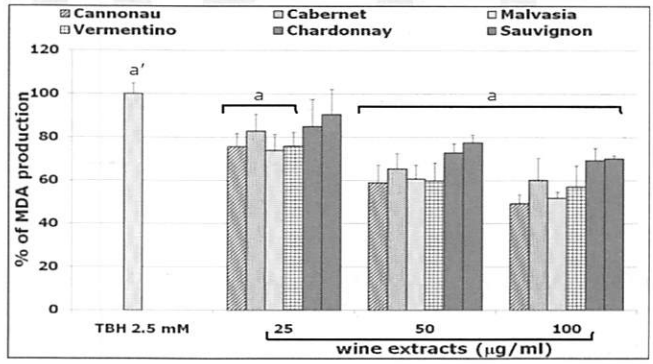

Figure 3. MDA production measured in the cells supernatants, pretreated with the wine extracts and treated with TBH 2.5mM. à=p<0.001 vs. controls, a=p<0.001 vs. TBH treated

Discussion

All the tested extracts exerted a significant antioxidant action, inhibiting the lipid peroxidation process both in chemical systems and in intestinal cells. The extracts from native cultivars, showed higher efficacy.
The activity of the phenolic extracts seems to be proportional to the total phenolic content but also to the relative proportions between the different classes of compounds and it is the result of an action of synergy between the various constituents.

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