Polyphenolic compounds of red wine: relatioship with the antioxidant properties and effects on the metabolic syndrome induced in high-fructose fed rats

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KEY WORDS: Antioxidant Capacity, Polyphenols, Metabolic Syndrome, Red Wine, Sprague-Dawley rats

Abstract

Epidemiologists have observed that a diet rich in polyphenolic compounds may provide a positive effects due to their antioxidant properties. Red wine is an excellent source of polyphenolic compounds. Objective of this work is a review of the polyphenolic compounds of red wine. The first study evaluates the antioxidant properties of Sicilian red wines in relatioship with their polyphenolic composition; the second investigates the corrective effects of some phenolic molecules on the metabolic syndrome induced in high-fructose fed rats. The results show that: i) the antioxidant capacity (CA) is not linearly correlated with the total polyphenol concentrations, but it depends of the kind of the phenolic compounds and on their order of polymerization; ii) the treatment with gallic acid (GA) and mixed phenolic molecules normalized blood pressure; the GA and delphinidin (Dph) treatments restored the index of insulin resistance to normality. All treatments significantly normalized the production of superoxide anion and the expression of p22phox and gp91phox subunits.

Introduction

Epidemiological evidence have shown that components of fruits are beneficial to human health and contribute to the prevention of degenerative processes caused by oxidative stress (Kaur and Kapoor, 2001; Vinson *et al.*, 2001). The polyphenolic molecules are antioxidants against the free radicals and they show a physiologic role as well. Dietary intake of antioxidant compounds are inversely related to coronary heart disease (Hertog *et al.*, 1997) and they act as anti-ulcer, antispasmodic, antisecretory, or anti-diarrheal agents in the gastrointestinal tract (Carlo *et al.*, 1999). Moreover, regular moderate wine consumption has been shown to favourably influence several health factors associated with metabolic syndrome. Wine is very rich in polyphenolic compounds. The polyphenolic contents of wine consist in two classes *flavonoids and non-flavonoids* (Shahidi & Naczk, 1995). These compounds increase the plasma antioxidant capacity in the human body after red wine consumption (Serafini *et al.*, 1998). **Objective** of this work was investigate the properties of the polyphenolic compounds of red wine. The first study evaluates the antioxidant properties of different samples of Sicilian red wine in relationship with their polyphenolic composition; the second study explores the corrective effects of some phenolic compounds alone or mixed on the metabolic syndrome induced on male Sprague-Dawley rats fed with a fructose-enriched diet (Azay-Milhau *et al.*, 2007). The latter was conduct in cooperation with the University of Montpellier (France).

Materials and Methods

Study on red wine samples: experimental protocol and methods

In the first study (Di Majo *et al.*, 2008) have been analysed seventeen samples of red wine selected from different grape varieties grown in Sicily and with different vintages. All the samples shared the same vineyard location, cultivation system, climate, soil types, vine cultivation practices, harvesting time, production process and barrel aging. The vintages 2002, 2003, and 2004 have been taken into consideration. For each sample both the total polyphenolic contents and the antioxidant capacity were studied; in order to evaluate its influence on the antioxidant capacity (AC). The total phenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Di Majo *et al.*, 2008) while the AC was performed by crocin bleaching assay according to the protocol of Di Majo *et al.* (2008). The

phenolic molecules was carried out using an HPLC-DAD (Di Majo et *al.*, 2008).

Study on rats: experimental design and methods

Total of 63 male Sprague-Dawley rats weighing 185-220 g were provided from *Iffa-Credo* (*Larbresle*, Francia). They were subdivided into 7 groups of homogeneous weights of nine animals each: a) control (**C**) fed a standard chow; b) fructose-fed (**F**); c) catechin and fructose-fed (**FC**); d) gallic acid and fructose-fed (**FAG**); e) resveratrol and fructose-fed (**FR**); f) delphinidin and fructose-fed (**FD**); g) mixed molecules and fructose-fed (**FMix**). Standard chow contained 60%vegetable starch, 11% fat, and 29% protein,whereas fructose-fed rats received a diet containing 66% fructose, 22% proteins, and 12% fat. Rats received polyphenol compounds once daily by gavage for 6 weeks. C and F groups received water only. The mixture was constituted from four compounds (catechin, gallic acid, delphinidin, resveratrol) at 25%. Each group received 10mL/Kg of solution. The parameters evaluated were: blood pressure; glucose, insulin, index of insulin resistance, HDL cholesterol (HDLC), total cholesterol (TC), triglycerides (TG), and phospholipids (PL); superoxide anion production, Advanced Oxidation Proteins Products and over expression of NADPH oxidise subunits (p22phox and gp91phox). The study protocol was approved by the hospital supervisory committee for animal studies and was carried out in adherence to their guidelines. Blood pressures were assessed with a electrosphygmomanometer (Chakir et *al.*, 1998). Insulin was evaluated by radioimmunoassay. The degree of insulin resistance was estimated at the baseline by Homeostatic Model Assessment (HOMA-ir) according to the method described by Matthews et *al.* (1985). The glucose concentration was evaluated by spectrophotometric method (Trinder, 1969). HDLC, TC, TG, and PL were determined using a Konelab automatic plasma analyzer. Superoxide anion production was evaluated in tissues by chemiluminescence; the analysis of over expression of NADPH oxidise subunits (p22phox and gp91phox) by Immunoblotting (Al-Awwadi et *al.*, 2005).

Results and Discussion

Antioxidant properties of red wine in relatioships with its polyphenolic composition

The study on seventeen samples of Sicilian red wine show that the antioxidant capacity (CA) is not linearly correlated with the total polyphenol concentrations (**Table I**), but it depends of the kind of the phenolic compounds and on their order of polymerization;.

Some of these samples, such as Cabernet-Sauvignon 2004 (pair 1 and 2) or Syrah 2004 (pair 5 and 6); Nero D'Avola 2003 (pair 9 and 10), Syrah 2002 (pair 16 and17), derive from the same grape variety and vintage but they belong to different production lots

Furthermore, the antioxidant capacity of red wines tested appears to be largely influenced by cathechin, myricetin,

Table I – Antioxidant capacity and total phenolic concentrations in Sicilian red wines from different varieties of grape, produced in different vintage years.

N°	Variety of grapes	Vintage years	Antioxidant Capacity (mM TRE)	Total polyphenols (g/L GAE)
1	Cabernet-Sauvignon	2004	1.40±0.16	2.72 ± 0.41
2	Cabernet-Sauvignon	2004	1.511 ± 0.35	2.38 ± 0.03
3	Merlot	2004	4.07 ± 0.10	2.999 ± 0.312
4	Nero D'Avola	2004	2.30 ± 0.26	2.87 ± 0.07
5	Syrah	2004	1.47 ± 0.62	3.02 ± 0.04
6	Syrah	2004	1.20 ± 0.21	3.00 ± 0.01
7	Cabernet-Sauvignon	2003	5.55 ± 0.50	3.58 ± 0.01
8	Merlot	2003	4.86 ± 0.42	3.36 ± 0.06
9	Nero D'Avola	2003	1.42 ± 0.34	3.55 ± 0.18
10	Nero D'Avola	2003	1.29 ± 0.43	3.73 ± 0.07
11	Sangiovese	2003	2.32 ± 0.40	2.36 ± 0.19
12	Syrah	2003	1.69 ± 0.09	3.41 ± 0.15
13	Cabernet-Sauvignon	2002	3.03±0.03	2.95 ± 0.05
14	Merlot	2002	2.22 ± 0.75	3.31 ± 0.12
15	Nero D'Avola	2002	0.65 ± 0.54	2.36 ± 0.05
16	Syrah	2002	5.73 ± 0.11	3.05 ± 0.06
17	Syrah	2002	5.83 ± 0.23	3.12 ± 0.35

Some of these samples, such as Cabernet-Sauvignon 2004 (pair 1 and 2) or Syrah 2004 (pair 5 and 6) Nero D'Avola 2003 (pair 9 and 10), Syrah 2002 (pair 16 and17), derive from the same grape variety and vintage but they belong to different production lots

gallic acid and peonidin-3-O-glucoside levels, while the other compounds gave the lowers correlations (**Table 2**)

Table 2 - correlation between single phenolic compounds and antioxidant capacity values of red wines

Polyphenolic compounds	r2
Catechin	0.54
Epicatechin	0.45
Quercetin	0.16
Myricetin	0.53
Gallic Acid	0.55
Cyanidin	0.20
Cyanidin-3-O-glucoside	0.38
Peonidin	0.11
Peonidin-3-O-glucoside	0.54
Delphynidin	0.46
Malvidin-3-O-glucoside	0.29

(r2) represents the correlation coefficient between antioxidant capacity values and content of single phenolic compounds (Microsoft Office Excel 2003)

Effect of polyphenolic compound on the metabolic syndrome induced in high-fructose fed rats

The date of this work indicate that the blood pressure, the degree of insulin resistance (HOMA-ir), the superoxide anion production in the heart or in the aorta and the synthesis of the gp91and p22phox NAD(P)H subunits were higher in the rats fed with a fructose enriched-diet with respect to traditional diet (control group). In the current study we showed a) that only the treatment with gallic acid and mixed phenolic molecules normalized blood pressure (**Table 3**); b) that blood glucose and insulin were not significantly differente between C and F groups while the index of insulin resistence is reduced to normality with the gallic acid and delphinidin treatments (**Table 3**); c) that all treatment significantly reduced the production of superoxide anion and normalized the expression of p22phox and gp91phox subunits (**Table 3**); d) that the lipid profile was higher in F group with respect to C group and all treatments were not able to restored it to normality (**Table 4**).

Conclusion

The polyphenolic of red wine are very interesting compounds but other study are necessary to prove the efficacy of this molecules especially in vivo; furthermore future studies will determine the respective role and mechanism of the various polyphenolic families to prevent the disorders associated to metabolic syndrome.

Table 3 - Metabolic and markers of oxidative stress of the various treatment groups

Parameters	C	F	FC	FAG	FR	FD	FMix
Blood Pressur (mmHg)	121,7±2,3	133,7±4,2*	140,0±8,1	119,5±5,3**	126,7±3,6	133,3±3,9	116,0±2,9
HOMA-ir	22 ±2,6	32,6± 4,8*	30,1±5,3	18,1±3,1**	23,1±5,0	20,8±3,2**	26,1±6,0
Glucose (mM)	6,2 ± 0,3	6,9± 0,3	6,2 ± 0,4	5,9± 0,4	6,2 ± 0,4	6,3± 0,5	6,9 ± 0,5
Insuline (ng/ml)	3,4± 0,4	4,1 ± 0,6	4,3 ± 0,8	2,7 ± 0,2	3,3 ±0,7	2,9 ±0,4	3,1±0,7
Superoxide anion (mV/mg)	105,4±4,8	146,5±7,7*	95,9±8,8**	102,0±11,7**	106,7±11,2**	96,3±9,3**	106,6±14,6**
NADPH oxidase	423±51	788±91*	543±59**	539±48**	550±122**	525±71**	719±87**
p22phox (AU)							
NADPH oxidase	939±101	5151±1131*	725±71**	477±50**	855±85**	754±74**	828±71**
gp91phox (AU)							

** p<0.05 from control ** p< 0,05 from fructose Values are means (±SEM) determined in control (C) fructose (F) fructose-catechin (FC) fructose-gallic acid (FAG) fructose-resveratrol (FR) fructose-delphynidin (FD) fructose-mix treated (Fmix) groups. HOMA-ir was computed with the formula: plasma glucose (mmol/L) times serum insulin (mU/L) divided by 22.5 (AU: arbitrary unit)*

Table 4 - Lipide profile of the various treatment groups

Parameters	C	F	FC	FAG	FR	FD	FMix
TG (g/L)	2,3±0,2	5,9±0,6 *	5,3±0,6*	4,4±0,4*	5,6±0,9*	4,8±0,5*	4,9±0,9*
PL (g/L)	2,1±0,1	2,9±0,1*	3,1±0,1*	2,8±0,1*	3,2±0,2*	2,9±0,1*	2,9±0,2*
TC (g/L)	1,9±0,1	2,3±0,1*	2,5±0,1*	2,4±0,1*	2,7±0,1*	2,6±0,1*	2,6±0,1*
HDL-C(g/L)	1,3±0,1	0,8±0,1*	0,9±0,2*	0,9±0,1 *	0,9±0,1*	1,0±0,1	1,0±0,1
Non-HDL-C (g/L)	0,7±0,1	1,7±0,1*	1,6±0,2*	1,5±0,1*	1,9±0,2*	1,6±0,1*	1,6±0,2*

** p<0.05 from control Values are means (±SEM) determined in control (C) fructose (F) fructose-catechin (FC) fructose-gallic acid (FAG) fructose-resveratrol (FR) fructose-delphynidin (FD) fructose-mix treated (Fmix) groups.*

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