SIMULTANEOUS DETERMINATION OF PHENOLIC COMPOUNDS IN SELECTED ITALIAN RED WINES

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Abstract

Over the years, pathological implications have often been remarked in the free radicals formation in the human body, underlining how a diet rich in antioxidant substances can be able to limit oxidative damage. Free radicals are extremely harmful to living organisms to do they attack different constituents cell, which leads to acceleration of the ageing process and sometimes, even, cell destruction or, if the DNA is involved, irreversible malfunctions. Nowadays, a wide range of food and drink, regularly consumed, have a high antioxidant capacity. So, a balanced and sustained intake of these substances with antioxidant characteristics is recommended in the diet. Grapes and grape products, particularly red wine, are a rich source of antioxidant compounds that, as well as their influence to the colour and organoleptic properties, have also beneficial to human health.

Scientific reports show that the antioxidant potential of wine is closely related to its content in phenolic compounds. The phenolic composition in wine is affected by a number of factors, including grape variety, fermentation processes, vinification techniques, aging and geographical and environmental factors (soil type and climate).

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In this work, the selected italian red wines of the appellation of origin (DOC) were assayed for their polyphenolic content. The total polyphenols content (TPC) was determined by the method of Folin-Ciocalteau. The concentration of eight individual phenolic compounds was determined using a reversed-phase performance liquid chromatography (HPLC) with UV/Vis detector.

Riassunto

Nel corso degli anni sono state più volte evidenziate implicazioni patologiche causate dalla formazione di radicali liberi nel corpo umano, sottolineando come una dieta ricca di sostanze antiossidanti può essere in grado di limitarne i danni ossidativi. I radicali liberi sono estremamente nocivi agli organismi viventi in quanto attaccano i diversi componenti cellulari con conseguente accelerazione del processo di invecchiamento, talvolta morte cellulare o addirittura, se coinvolto il DNA, danni irreversibili. Una vasta gamma di alimenti e bevande, oggi consumate regolarmente, possiedono un'elevata capacità antiossidante, pertanto un dosaggio equilibrato e prolungato di sostanze con tali caratteristiche è raccomandato nella dieta. L'uva ed i prodotti da essa derivati, in particolare il vino rosso, sono una ricca fonte di composti antiossidanti che, oltre ad influenzarne il colore e le proprietà organolettiche, esercitano azione benefica per la salute dell'uomo. Dati scientifici hanno dimostrato infatti, che il potenziale antiossidante del vino rosso è strettamente correlato al contenuto in composti fenolici.

La componente fenolica di un vino è influenzata da una serie di fattori, qualità dell'uva, processi di fermentazione, tecniche di vinificazione, invecchiamento e da fattori ambientali e geografici (caratteristiche del terreno, condizioni climatiche).

Nel presente lavoro è stata valutata la composizione fenolica di campioni di vino rosso italiani selezionati a denominazione di origine controllata (DOC). Il contenuto totale di fenoli (TPC) è stato determinato mediante il metodo di Folin-Ciocalteu. La concentrazione di otto composti fenolici mediante cromatografia liquida ad alte prestazioni (HPLC) a fase inversa con rivelatore UV/Vis.

Keywords: polyphenols, HPLC, TPC, red wines

Introduction

Over the years, many works has been carried out on the beneficial effects of phenolic compounds as antioxidant molecules, which help to neutralize free radicals (1-5). Free radicals are extremely harmful for

living organisms as they attack different constituents of the cell. This leads the acceleration of the ageing process and sometimes cell destruction or, if the DNA is affected, irreversible malfunctions (6). Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidant activity of phenolic compounds is essentially due to the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to a free radical and the ability of the phenolic moiety to support an unpaired electron due to delocalization around the π-electron system (7).

Many studies, furthermore, have shown the antioxidative properties of phenolic compounds in protection against atherosclerotic pathologies and heart diseases (8-12). Other effects include modulation of eicosanoid synthesis toward a more antiatherogenic pattern and the polyphenols have been associated with a reduction of the risk of cancer (13).

The phenols are substances naturally occurring in fruit, vegetables and other nutrients of the human diet and it are characterized by the presence of one or more benzene-type rings. Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerised compounds (14). Despite this structural diversity, the group of compounds is often referred to as polyphenols.

Grapes and grape products such as wine, particularly red wine, are a rich source of antioxidant phenolic compounds. The active phenolic compounds identification, responsible for red wine's antioxidant properties, has raised much interest (15-16). These properties provide a rationale for exploring the polyphenolic content of commercial wines to define those that are especially abundant and to stimulate the development of enological techniques for their enrichment (17).

The literature reports several studies in which the polyphenolic compounds in wines are mainly determined using chromatographic techniques with UV or DAD (Diode Array Detector) detector. Many other works have also involved the use of liquid chromatography coupled with mass-spectrometry. These techniques are very expensive and consequently not widely used in routine analyses.

In this work, the selected italian red wines of the "appellation of origin" were assayed for their polyphenolic content. DOC wines are produced in specific well-defined regions according to specific rules designed to preserve the traditional wine-making practices of the individual regions.

Therefore, a wine has to meet certain quality standards to be qualify as DOC.

The total polyphenols (TPC) was determined by the method of Folin-Ciocalteau. To identify individual phenolic compounds, a recently developed method based on liquid chromatographic system, was used (18). The procedure, described in *materials and methods* section, foresee the use of equipments easily available in most laboratories, so it is suitable for routine analyses.

Experimental

Materials and methods

Chemicals: methanol was supplied by Merck (Darmstadt, Germany), acetic acid by Carlo Erba (Milano, Italy) and water was previously purified in a Mili-Q system (Millipore, Bedford, MA, USA). Standards of caffeic acid (99%), (±)-catechin hydrate, chlorogenic acid, cinnamic acid, (-)-epicatechin, ferulic acid, gallic acid, trans-resveratrol (Figure 1) and the sodium carbonate were acquired from Sigma (St.Louis, MO, USA). FC reagent was supplied by Fluka.

The analyzed compounds belong to the following families:

- flavonoids (catechin and epicatechin);
- phenolic acids (caffeic, chlorogenic, cinnamic, ferulic and gallic acid);
- derivatives of phenolic acids such as stilbenes (*trans*-resveratrol).

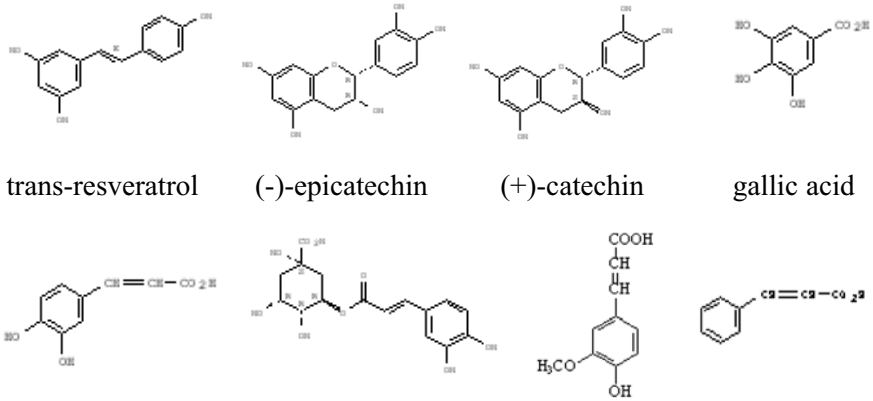

Fig. 1 – Chemical structure of the phenolic compounds.

Typology of samples subject to experimentation: a group of red wines with appellation of origin (DOC) was analyzed. The samples of different vintages were selected from several areas of Italy: Negro Amaro, Nero d'Avola, Assisi Rosso, Chianti, Montepulciano. The samples were filtered through a PTFE 0.45-µm membrane filter before the spectrophotometric analysis and the chromatographic determination. The samples were immediately analyzed after the bottle opening.

Equipments: the concentration of individual compounds was determined by liquid chromatography apparatus (Shimadzu Series VP, Kyoto, Japan) equipped with a control system mod. FCV-10ALVP, two reciprocating plungers (mod. LC-10ATVP), a solvent degasser (DGU-148), a rheodyne injection system (mod. 7725i) with a 20 µL loop, coupled to a spectro-Monitor L-4250 UV/Vis detector, Merck-Hitachi (Tokio, Japan). The analytical column was SupelcosilTM LC-18 (150 x 4.6 mm, 3 i.d. mm, Bellefonte, PA) in conjunction with a SupelguardTM LC-18 (2 x 2.1 mm) guard cartridge column. Data acquisition was performed by an interface model Data Apex CSW32 (Prague, Czech). The spectrophotometric measurements were performed by UV/Vis spectrophotometer 554 Perkin-Elmer, Germany.

Chromatographic procedure: all the polyphenol standard solutions were prepared in methanol and stored at 4ºC in dark conditions, until their use. The standard solutions were prepared at least once at week. All the analyses were carried out in triplicate and in gradient mode after filtration. The polyphenol compounds, were determined using the optimized method described in a previously work (18). The procedure was performed with a gradient elution using 5% acetic acid in methanol (solvent A) and 5% acetic acid aqueous solution (solvent B) as eluent. The initial conditions were 21% A and 79% B at a flow rate of 0.4 mL/min. After 5 min, 27% A and 73% B at a flow rate of 0.5 ml/min with a linear gradient in 1 min. At 15 min solvent B 45% at the same flow rate until termination of the run. This was followed by a 10 min equilibrium period with the zero time solvent mixture before to injection of the next sample. The eluted compounds were monitored at wavelength, 280 nm for (±)-catechin, (-)-epicatechin, gallic and cinnamic acid, 306 nm for trans-resveratrol and ferulic acid and 330 nm for chlorogenic and caffeic acid. The compounds concentration in red wines were calculated by external standard method.

Determination of total phenolic content (TPC): the TPC was determined by the FC reagent (19), using gallic acid as standard. This method is based on the reduction of a phosphowolframate-phophomolybdate

complex by phenolic compounds to blue reaction products. To 20 µL of wine sample (diluted 1:5), introduced into a test tube, were added 1.58 mL of water and 100 µL of FC reagent. After 8 minutes 300 µL of sodium carbonate solution 20% was added and the solution was diluted to 10 mL with Milli-Q water. The sample absorbance was measured at 765 nm after 30 min of reaction at 40 ºC. The results were expressed as mg of gallic acid equivalents (GAE) L^{-1}. For the calibration curve preparation, 20 µL aliquots of 150, 250, 500 and 650 mg/L of aqueous gallic acid solutions were handled with the same procedure of the wine samples. The determinations were all performed in triplicate.

Results and discussion

The aim of this study was to determine a class of antioxidant compounds that could be present in grapes and consequently in grape products as wine, and to evaluate the wine total phenolic content, in order to increase quality standards of a beverage commonly consumed in our country. All samples, after the bottle opening, were divided in two aliquots, one for the TPC determination, and the other part for the HPLC/UV analyses. Under elution conditions, an optimal resolution of eight phenolic compounds was achieved using a chromatographic method already optimized for other samples and antioxidant molecules.

Table 1 summarizes the mean values and standard deviations of three replicate determinations. The table shows catechin and epicatechin as the two most abundant flavonoid found in the analyzed samples, with concentration ranged from 98.31 to 181.19 mg/L and from 53.90 to 102.25 mg/L, respectively. The concentration values of phenolic acids were less than catechins, except for ferulic acid with a maximum value of 0.23 mg/L. Significant concentrations of cinnamic acid were also found in all samples (from 72.98 to 196.84 mg/L). The trans-resveratrol concentrations ranged from 0.74 to 1.74 mg/L, which were higher in younger wine samples regardless of the cultivar type.

As seen from the above values and in accordance with other authors, there is a considerable variability in the wines phenolic concentrations. This variability occurs in samples produced by the same grape variety as well as from different vintage.

The results obtained on some analyzed samples were reported in Table 1 and in Figure 2.

The total phenolic content (TPC) was determined by spectrophotometric measurements with the calibration curve of gallic acid and it was expressed in mg/L. The results ranged from 2,015 (Negro Amaro, Puglia) to 2,650 mg/L (Chianti, Toscana) showing substantial differences for the investigated red wines. Table 1 summarizes the mean values of three replicate determinations. Our results are in agreement with those available in literature.

Our findings reflect the great and known phenolic composition variability in wines. The differences in the phenolic content could be due to a high intrinsic ability of some grape varieties to synthesize these antioxidant molecules. Therefore, the territory of origin, the composition of terrain, the type and amount of fertiliser utilized and the climate, become decisive factors. Moreover, the wine phenolic content is influenced also by fermentation, the wine making process, technological treatments and the process of aging.

The FC method, such as methods for antioxidant activity determination, is based on the compounds redox properties. Therefore, the TPC values could partially express the antioxidant activity. The examined wines showed significant high phenolic levels and consequently high antioxidant power.

In conclusion, the analytical method carried out in this work optimizes chromatographic conditions to reach a good separation of eight phenolic wine constituents. This HPLC procedure is able to process a large number of samples. The described method, involving direct injection into HPLC, proved to be simple, rapid and reliable and it can be used especially for routine analyses.

The work could continue with the determination and the quantification of other antioxidant molecules and the analyses of other wine typologies. Would also be interesting finding possible analogies between the different cultivar and vintage and the study could be conducted towards the determination of total antioxidant activity (TAA) in all the samples analyzed to verify the possible correlation with the total phenolic content (TPC).

TABLE 1

CONCENTRATION OF POLYPHENOLS (mg/L ± SD) AND TPC VALUES EXPRESSED AS mg OF GALLIC ACID EQUIVALENTS (GAE) L^{-1} IN RED WINE SAMPLES

Samples	Negro Amaro[a]	Nero D'Avola	Assisi Rosso[b]	Chianti[c]	Montepulciano
Origin/Year	Puglia 2005	Sicilia 2006	Umbria 2006	Toscana 2006	Abruzzo 2005
Caffeic acid	7.44 ± 1.1	5.33 ± 1.3	20.53 ± 2.1	5.04 ± 1.3	3.86 ± 0.6
Catechin	98.31 ± 2.4	103.78 ± 1.4	149.96 ± 2.3	181.19 ± 1.5	178.37 ± 3.3
Chlorogenic acid	32.33 ± 3.1	17.58 ± 1.1	23.75 ± 1.3	15.19 ± 1.7	29.84 ± 1.6
Cinnamic acid	72.98 ± 2.8	83.40 ± 1.7	196.84 ± 4.0	156.42 ± 2.2	74.67 ± 2.5
Epicatechin	99.25 ± 1.9	102.25 ± 2.4	69.10 ± 2.3	53.9 ± 1.6	93.81 ± 2.3
Ferulic acid	0.19 ± 0.7	0.14 ± 0.4	0.16 ± 1.2	N.D.	0.23 ± 1.1
Gallic acid	22.71 ± 1.7	29.81 ± 1.5	22.71 ± 0.8	21.29 ± 1.3	25.55 ± 1.4
***trans*-Resveratrol**	0.85 ± 0.2	0.99 ± 0.3	1.24 ± 0.3	0.94 ± 0.2	1.74 ± 1.1
TPC	2,015	2,100	2,550	2,650	2,160

N.D.: Not Detected

a Negro Amaro-Malvasia Nera-Sangiovese

b Montepulciano-Merlot

c Sangiovese 75%-Canaiolo Nero 10%- Trebbiano Toscano-Malvasia

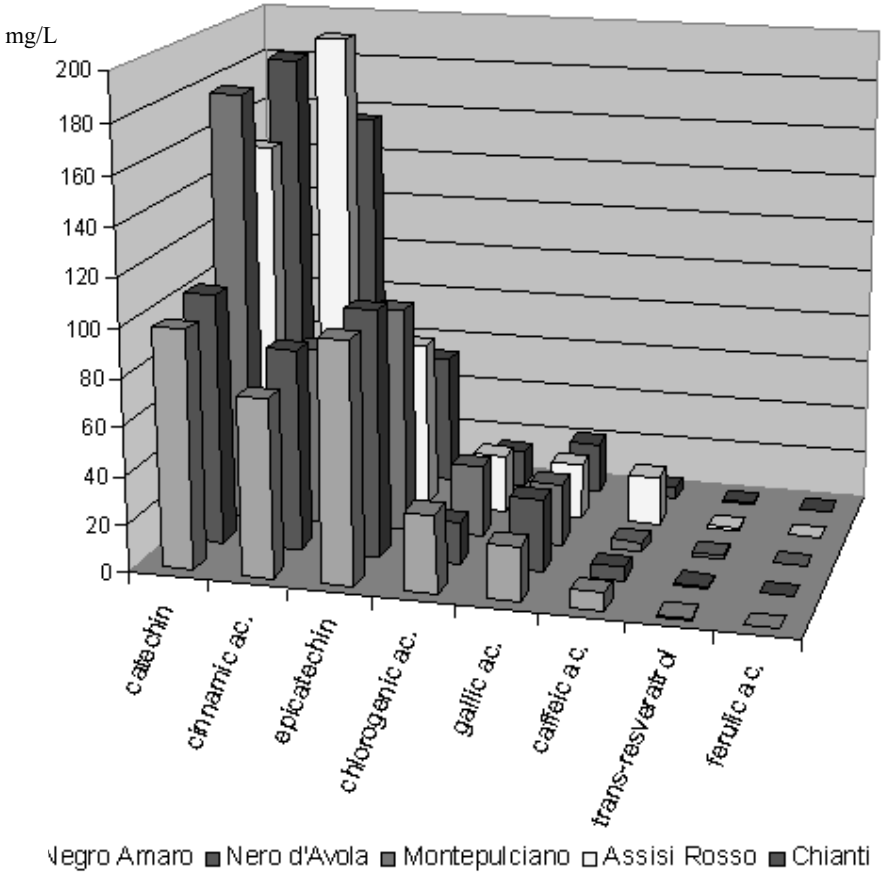

Fig. 2 – Concentration values (mg/L) of the eight phenolic compounds in the sample wines analyzed

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REFERENCES

(1) A. GHISELLI, M. NARDINI, A. BALDI, C. SCACCINI, "Antioxidant activity of different phenolic fractions separated from an Italian red wine", *J. Agric. Food Chem. 1997*, 46, 361-367.

(2) I. TEDESCO, M. RUSSO, P. RUSSO, G. IACOMINO, G. L. RUSSO, A. CARRATURO, C. FARUOLO, L. MOIO, R. PALUMBO, "Antioxidant effect of red wine polyphenols on red blood cells", *J. Nutr. Biochem. 2000*, 11, 114-119.

(3) M. S. FERNANDEZ-PACHON, D. VILLANO, M. C. GARCIA-PARILLA, A.M. TRONCOSO, "Antioxidant activity of wines and relation with their polyphenolic composition", *Anal. Biochem. Acta 2004*, 513, 113-118.

(4) D. VILLANO, M. S. FERNANDEZ-PACHON, M. L. MOYA', A. M. TRONCOSO, M. C. GARCIA-PARILLA, "Radical scavenging ability of polyphenolic compounds towards DPPH free radical", *Talanta 2007*, 71, 230-235.

(5) D. RIVERO, S. PEREZ-MAGARINOS, M. L. GONZALES-SANJOSE', V. VALLS- BELLES, P. CODONER, P. MUNIZ, "Inhibition of induced DNA oxidative damage by beers: correlation with the content of polyphenols and melanoidins", *J. Agric. Food Chem*. *2005*, 53, 3637-3642.

(6) N. S. DE GAULEJAC, Y. GLORIES, N. VIVAS, "Free radical scavenging effect of anthocyanins in red wines", *Food Research International 1999*, 32, 327-333.

(7) J. KANNER, E. FRANKEL, R. GRANIT, B. GERMAN, E. KINSELLA, "Natural antioxidants in grapes and wines", *J. Agric. Food Chem*. *1994*, 42, 64-69.

(8) R. ESTRUCH, "Wine and cardiovascular disease", *Food Research International 2000*, 33, 219-226.

(9) C. SANTOS-BUELGA, A. SCALBERT, "Proanthocyanidins and tannin-like compounds-nature, occurrence, dietary intake and effects on nutritional and health", *Journal of the Science of Food and Agriculture 2000*, 80, 1094-1117.

(10) I. STUPANS, A. KIRLICH, K. L. TUCK, P. J. HAYBALL, "Comparison of radical scavenging effect, inhibition of microsomal oxygen free radical generation, and serum lipoprotein oxidation of several natural antioxidants",

Journal of Agricultural and Food Chemistry 2002, 50, 2464-2469.

(11) A. Y. SUN, A. SIMONYI, G. Y. SUN, "The "French paradox" and beyond: Neuroprotective effects of polyphenols", *Free Radical Biology and Medicine 2002*, 32, 314-318.

(12) F. VISIOLI, L. BORSANI, C. GALLI, "Diet and prevention of coronary heart disease: the potential role of phytochemicals", *Cardiovascular Research 2000*, 47, 419-425.

(13) G. J. SOLEAS, J. DAM, M. CAREY, D. M. GOLDBERG, "Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines", *Journal of Agriculture and Food Chemistry 1997*, 45, 3871-3880.

(14) L. BRAVO, "Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance", *Nutrition Reviews 1998*, 56, 317-333.

(15) N. L. KERRY, M. ABBEY, "Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation in vitro", *Atherosclerosis 1997*, 135, 93-102.

(16) GOBBI L., NICOLETTI I., TAROLA A.M., CORRADINI C., "Evaluation of the level of cis- and trans-resveratrol and their glucosides in red wines produced il Lazio region (area)", *Journal of Commodity Science 2004*, 43, 173-190.

(17) G. J. SOLEAS, J. DAM, M. CAREY, D. M. GOLDBERG, "Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines", *Journal of Agriculture and Food Chemistry 1997*, 45, 3871-3880.

(18) A.M. TAROLA, F. MILANO, V. GIANNETTI, "Simultaneous determination of phenolic compounds in red wines by HPLC-UV", *Analytical Letters 2007*, 40, 2433-2445.

(19) R. C. MINUSSI, M. ROSSIC, L. BOLOGNA, L. CORDI, D. ROTILIOC, G. M. PASTOREA, N. DURAN, "Phenolic compounds and total antioxidant potential of commercial wines", *Food Chemistry 2003*, 82, 409-416.