Journal of Food Composition and Analysis 21 (2008) 589–598

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Article

Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, *in vitro* antioxidant activity and interactions

P. Iacopini [a,*], M. Baldi [b], P. Storchi [c], L. Sebastiani [a]

[a] *Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy*
[b] *CRA, Istituto Sperimentale di Enologia, SOP Gaiole in Chianti, Via di Vertine 1, 53013 Gaiole in Chianti Siena, Italy*
[c] *CRA, Istituto Sperimentale di Viticoltura, SOP Arezzo, Via Romea 53, 52020 Arezzo, Italy*

ARTICLE INFO

Article history:
Received 17 December 2007
Received in revised form
13 March 2008
Accepted 19 March 2008

Keywords:
Antiradical capacity
DPPH
Grape seeds
Grape skin
Peroxynitrite
Tyrosine nitration
Vitis vinifera

Abbreviations:
3-NT, 3-nitro-tyrosine
DM, dry matter
DPPH•, 2,2-diphenil-1-picridazil
GAE, gallic acid equivalent
LDL, low density lipoprotein
ME, malvidin-3-*O*-glu equivalents
$ONOO^-$, peroxynitrite

ABSTRACT

The extracts obtained from skin and seeds of 10 native Tuscan and international *Vitis vinifera* varieties were evaluated for their antioxidant activity, total phenolic and anthocyanin content and subjected to HPLC–UV analysis to quantify the content of five phenolic constituents of biological interest: catechin and epicatechin in seeds and quercetin, rutin and resveratrol in skin extracts. The antioxidant activity of the extracts and pure compounds was assessed by means of two different *in vitro* tests: scavenging of the stable DPPH• radical and of authentic peroxynitrite ($ONOO^-$). All the extracts showed significant antiradical capacity: Merlot skin was most active towards both radicals. All the five phenols investigated possessed strong antiradical activity. Quercetin, catechin and epicatechin showed maximum activity (respectively, $IC_{50(DPPH•)}$ 5.5, 6.7 and 6.8 μM, $IC_{50(ONOO^-)}$ 48.8, 55.7 and 56.7 μM). Potential antiradical interactive effects among the five compounds were also investigated and results indicated possible synergy between quercetin, rutin and resveratrol towards $ONOO^-$. The effect was additive for catechin and epicatechin.

1. Introduction

The consumption of fruits plays an important role as an health-protecting factor. This beneficial effect is mainly associated with the antioxidant activity of the phenolic compounds which are largely present in fruits and in beverages made from them. These effects are due to the properties of antioxidants to act as reducing agents by donating hydrogen, by quenching singlet oxygen, by acting as chelators and by trapping free radicals. These highly reactive molecules are present in biological systems and may oxidize nucleic acids, proteins, lipids, which may initiate degenerative diseases such as cancer, hearth disease, dermal disorders and aging (Cook and Samman, 1996; Harborne and Williams, 2000; Heim et al., 2002). The antioxidants can reduce this risk. Fruits are one of the most important sources of antioxidants such as vitamins and phenolic phytochemicals. The antioxidant activity of dietary polyphenols is considered to be much greater than that of the essential vitamins, therefore contributing significantly to the health benefits of fruits (Tsao and Yang, 2003).

In the past few years there has been an increasing interest in determining relevant dietary sources of antioxidant phenolics. Grape (*Vitis vinifera*) is among the fruits with the highest content of these compounds. A large amount of different phenolic compounds is present in skin, pulp and seeds, and they also undergo partial extraction during winemaking processes (Revilla and Ryan, 2000). Phenolics are divided into two groups: flavonoid (anthocyanins, flavan-3-ols, flavonols) and non-flavonoid compounds (hydroxybenzoic and hydroxycinnamic acids, stilbenes). Every family of polyphenols is directly responsible for the special characteristics of specific grapes varieties and the resulting wine. Anthocyanins are important polyphenols in the red grape skin,

* Corresponding author. Tel.: +39 050 883111; fax: +39 050 883495.
E-mail addresses: p.iacopini@sssup.it (P. Iacopini), isen.si@entecra.it (M. Baldi), paolo.storchi@entecra.it (P. Storchi), luca.sebastiani@sssup.it (L. Sebastiani).

0889-1575/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2008.03.011

while flavan-3-ols are the major polyphenols in the seeds (Bourzeix et al., 1986; Cheynier and Rigaud, 1986; Makris et al., 2006).

In the polyphenolic pool of red grape skin and seeds, there are some secondary compounds important for their antioxidant activity: catechin and epicatechin (flavan-3-ols), quercetin and its glycoside rutin (flavonols), and *trans*-resveratrol (stilbene). They are proven to be potent antioxidants and to have important biological, pharmacological and medicinal properties. All five compounds are shown to protect human low density lipoprotein (LDL) against oxidation more efficiently than α-tocopherol on a molar basis, acting as cardio protective agents (Frankel et al., 1993; Frankel et al., 1995; Meyer et al., 1998; Yilmaz and Toledo, 2004). Resveratrol and quercetin inhibit human platelet aggregation *in vitro* and exhibit potential anticancer properties, the first one by inducing cell differentiation (Frémont, 2000), the second one, as its glycoside, by inhibiting protein-tyrosine kinase (Sakkiadi et al., 2001). Finally resveratrol is able to mediate anti-inflammatory processes mainly inhibiting the expression of cyclooxygenase-1 and 2 (COX-1 and 2) and hydroperoxidase functions. It also shows estrogenic properties towards different cell lines (Frémont, 2000; King et al., 2006).

Several methods have been proposed to evaluate the antioxidant activity of vegetal extracts and pure compounds and it is accepted that this effect depends on the environmental conditions and procedures. *In vitro* assays for the free radical scavenging capacity are commonly based on the inactivation of stable synthetic radicals, such as the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), first envisaged by Blois (Blois, 1958). Another interesting approach is the study of the peroxynitrite ($ONOO^{-}$) scavenging capacity. Peroxynitrite is nowadays considered one of the most relevant radical generator involved in pathophysiological and toxicological processes. This anion is a product of the reaction between nitric oxide and superoxide and is a potent and versatile oxidant. Its importance in biological systems is based on its powerful ability to react with almost all classes of biomolecules. In fact, while it is relatively stable under basic conditions, at physiological conditions it forms two radicals ($NO_2^{\bullet}$ and $OH^{\bullet}$) that induce lipid peroxidation, disruption of cellular structures, inactivation of enzymes and ion channels through protein oxidation and nitration, and DNA damages (Virág et al., 2003). All these actions contribute to the onset and maintenance of pathologies such as atherosclerosis, neurodegenerative diseases (Torreilles et al., 1999) and cardiovascular disorders (Wattanapitayakul et al., 2000). Scavengers of these deleterious radicals and compounds able to prevent the consequences of their reactivity can contribute to the maintenance of health or healing processes (Heijnen et al., 2001; Chericoni et al., 2005).

Although the literature abounds with reports about phenolic composition and antiradical activity of wine or grape seed samples, there are very few papers that report data about grape seeds and skin of the same sample. Moreover, native Tuscan *V. vinifera* varieties have been occasionally studied and there have been no reports so far about their phenolic content and antiradical potential, especially in comparison to well-known international and Italian varieties.

The present study was undertaken to provide information related to the free radical scavenging capacity of red grapes by using different methods and to relate the results to their phenolic profile. Native Tuscan, international and Italian red grape grown in the same location were analyzed for their free radical scavenging activity by using two different *in vitro* models: the $DPPH^{\bullet}$ assay and the inhibition of the tyrosine nitration by peroxynitrite. To our knowledge, this is the first time that grape extracts are tested to determine their ability to prevent *in vitro* peroxynitrite-induced formation of 3-nitro-tyrosine (3-NT), a biomarker of the oxidative stress (Althaus et al., 2000). Moreover, while it is well demonstrated that several purified phenolic compounds individually exert significant antioxidant activity in various *in vitro* assays, very few papers are available about any interactions between phenolic compounds (Meyer et al., 1998; Guarnieri et al., 2007). In recent papers (Lotito and Frei, 2004; Guarnieri et al., 2007), there is an increasing interest in understanding how phenolic compounds can interact and in providing new and better knowledge of the antioxidant effect of complex phytochemical mixtures such as grape extracts.

Therefore in the present work we investigated the antiradical activity of five biologically and pharmacologically active phenolic compounds present in grape seeds and skin extracts, focusing on the interactions that can be involved in the activity of mixtures of these phytochemicals.

2. Materials and methods

2.1. Chemicals

Gallic acid, catechin, epicatechin, resveratrol, rutin, quercetin, malvidin-3-*O*-glu, trolox, Folin–Ciocalteau reagent, tyrosine, 3-NT, 2,2-diphenil-1-picridazil ($DPPH^{\bullet}$ 90%) and HPLC grade methanol, acetic acid, ethanol were purchased from Sigma Aldrich (Milan, Italy). All chemicals were of analytical or higher grade and the aqueous solutions were prepared by using ultra-pure water purified by Milli-Q System (Millipore, Milan, Italy).

2.2. Plant material

Ten *V. vinifera* genotypes were chosen among seven grape varieties: three native Tuscan (Colorino del Valdarno COL N6 clone, Canaiolo Nero N8 clone, Foglia Tonda FT BRO1 clone), two international (Merlot R12 clone, Cabernet Sauvignon R5 clone) and five clones of two Italian varieties (Montepulciano AP MP1 clone and Sangiovese ISV RC1, F9 A5 48, ISV 2 and AP SG 1 clones). The investigation was performed on mature vines of the collection fields of the Experimental Institute of Viticulture in Arezzo (Italy). Grape samples were collected in triplicate during the 2005 harvest season. Berry fresh and dry weight, berry skin dry weight, seeds per berry and seed dry weight were estimated on hundred berries of each replicate. Dry weight was measured after oven-drying the samples at 60 °C until constant weight.

2.3. Preparation of grape skin and seed extracts

After harvest, berries were snipped from the cluster. The skin and the seeds from 40 berries were manually separated from pulp and extracted with ethanol:water:hydrochloric acid 0.12 M (70:29:1 v/v/v) for 4 h. The extracts were centrifuged for 10 min at 3300 rpm (2500 g), using a Sigma 302K Centrifuge from Bicasa (Milan, Italy), with a swing-out rotor. The precipitate was extracted again with the same solvent and made up to a final volume of 200 mL for skin and 100 mL for seeds. Extracts were stored at −20 °C and analyzed within a month after extraction.

Extracts were purified through a Sep-Pak Plus C-18 cartridges from Waters (Milan, Italy) before analyses. The cartridge was first conditioned with 4 mL of methanol, followed by 4 mL of water, and then loaded with 1 mL of extract appropriately diluted with 19 mL of Milli-Q water in order to reduce the ethanolic content. The loaded cartridge was first washed with 4 mL of water and then the compounds were eluted with 2 mL of methanol. This solution was finally injected into an HPLC system (described in section HPLC) after filtering through a 0.45 μm cellulose filter (Millipore).