ANALYTICA CHIMICA ACTA 630 (2008) 107–115

available at www.sciencedirect.com

ScienceDirect

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

Ultrasound extracted flavonoids from four varieties of Portuguese red grape skins determined by reverse-phase high-performance liquid chromatography with electrochemical detection

Ivana Novak[a,b], *Patricia Janeiro*[a], *Marijan Seruga*[b], *Ana Maria Oliveira-Brett*[a,*]

[a] *Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal*
[b] *Department of Applied Chemistry and Ecology, Faculty of Food Technology, J.J. Strossmayer University of Osijek, HR-31000 Osijek, Croatia*

ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form
30 September 2008
Accepted 1 October 2008
Published on line 14 October 2008

Keywords:
Reverse-phase high-performance liquid chromatography
Electrochemical detection
Flavonoids
Antioxidants
Red grape skins
Ultrasound extraction

ABSTRACT

Several flavonoids present in red grape skins from four varieties of Portuguese grapes were determined by reverse-phase high-performance liquid chromatography (RP-HPLC) with electrochemical detection (ECD). Extraction of flavonoids from red grape skins was performed by ultrasonication, and hydrochloric acid in methanol was used as extraction solvent. The developed RP-HPLC method used combined isocratic and gradient elution with amperometric detection with a glassy carbon-working electrode. Good peak resolution was obtained following direct injection of a sample of red grape extract in a pH 2.20 mobile phase. Eleven different flavonoids: cyanidin-3-*O*-glucoside (kuromanin), delphinidin-3-*O*-glucoside (myrtillin), petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside (oenin), (+)-catechin, rutin, fisetin, myricetin, morin and quercetin, can be separated in a single run by direct injection of sample solution. The limit of detection obtained for these compounds by ECD was 20–90 pg/L, 1000 times lower when compared with photodiode array (PDA) limit of detection of 12–55 ng/L. RP-HPLC–ECD was characterized by an excellent sensitivity and selectivity, and appropriate for the simultaneous determination of these electroactive phenolic compounds present in red grape skins.

1. Introduction

Plants contain high concentrations of phytochemicals with antioxidant properties, such as vitamin C, vitamin E, beta-carotene (which the body converts into vitamin A), and also polyphenols. Phenolic compounds constitute one of the most numerous and ubiquitously distributed groups of plant secondary metabolites [1,2]. Natural polyphenols can range from simple molecules (phenolic acids, stilbenes, flavonoids and others) to highly polymerized compounds (lignins, tannins, etc.), with flavonoids representing the most common and widely distributed sub-group, with more than 5000 phenolic structures currently known, and the list is constantly expanding. The basic structure of flavonoids consists of the tricyclic C_6–C_3–C_6 "flavan skeleton" (Fig. 1). According to differences in the number of hydroxyl groups, conjugation between the aromatic rings, nature, number and position of glycoside and methoxy substituent's attached to the aromatic

* *Corresponding author*. Tel.: +351 239 835295; fax: +351 239 835295.
E-mail address: brett@ci.uc.pt (A.M. Oliveira-Brett).
0003-2670/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2008.10.002

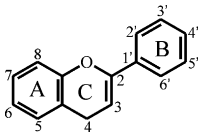

Fig. 1 – Structure of flavan skeleton.

rings, flavonoids can be divided into several subclasses, e.g. flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanins [2–4].

The interest in the bioactivity of phenolic compounds of higher plants is due, at least in part, to the potential health benefits of these polyphenolic components as well as to their involvement in important biological and industrial processes. It is well known that diets rich in fruits and vegetables protect against cardiovascular disease [5–7]. Beneficial health effects of polyphenols are believed to come from their antioxidant activity and capacity to protect important macromolecules, such as chromosomal DNA, structural proteins and enzymes, low-density lipoproteins and membrane lipids, from damage caused by the action of free radicals [8,9].

Many studies have been carried out on red grapes [10–12] and wines [10,11,13] that represent extremely rich sources of phenolic antioxidants. In red grapes flavonoids are mainly contained in the skin and seeds of grape berries. The main flavonoids contained in red grapes are flavan-3-ols, flavonols and anthocyanins (Fig. 2). Flavan-3-ols are located in both the grape skins and the seeds; however, skins contain much lower concentrations of flavan-3-ols than seeds and their composition is also different [10–12]. Grape skin flavanols are mainly in the form of polymers (tannins) and oligomers (procyanidin) of catechin and epicatechin, while monomer flavan-3-ols represent only a small proportion of total grape skin flavanols [14], with (+)-catechin being the most abundant. Flavonols are present in grape skins exclusively in the form of 3-*O*-glycosides, with glycoside derivatives of quercetin as the most abundant compounds from this sub-group of flavonoids. Glycosidic forms of myricetin, kaempferol and isorhamnetin have been identified [12,15,16].

One of the major groups of flavonoids found in grapes and wines are anthocyanins. Anthocyanins are glycosides and acylglycosides of anthocyanidins. The principal naturally occurring anthocyanins in red grapes are 3-*O*-glucosides of malvidin, delphinidin, peonidin, petunidin and cyanidin, with malvidin-3-*O*-glucoside being the most abundant in all *Vitis vinifera* L. varieties (Fig. 2). Anthocyanins are exclusively present in the cell walls and vacuoles of grape skins, and are directly responsible for the colour of grapes and of young red wines. Indirectly, through copigmentation reactions with other polyphenols, which result in the formation of stable complex polymers, they are responsible for the colour of final, matured wines [17]. Moreover, the anthocyanin composition of wine is proposed to be an indicator of wine authenticity [18,19].

Due to great importance of polyphenols for wine quality there is a growing interest in the development of selective and sensitive methods for their detection and quantification. Polyphenols are well known to be electroactive due to the presence of hydroxyl groups attached to the aromatic

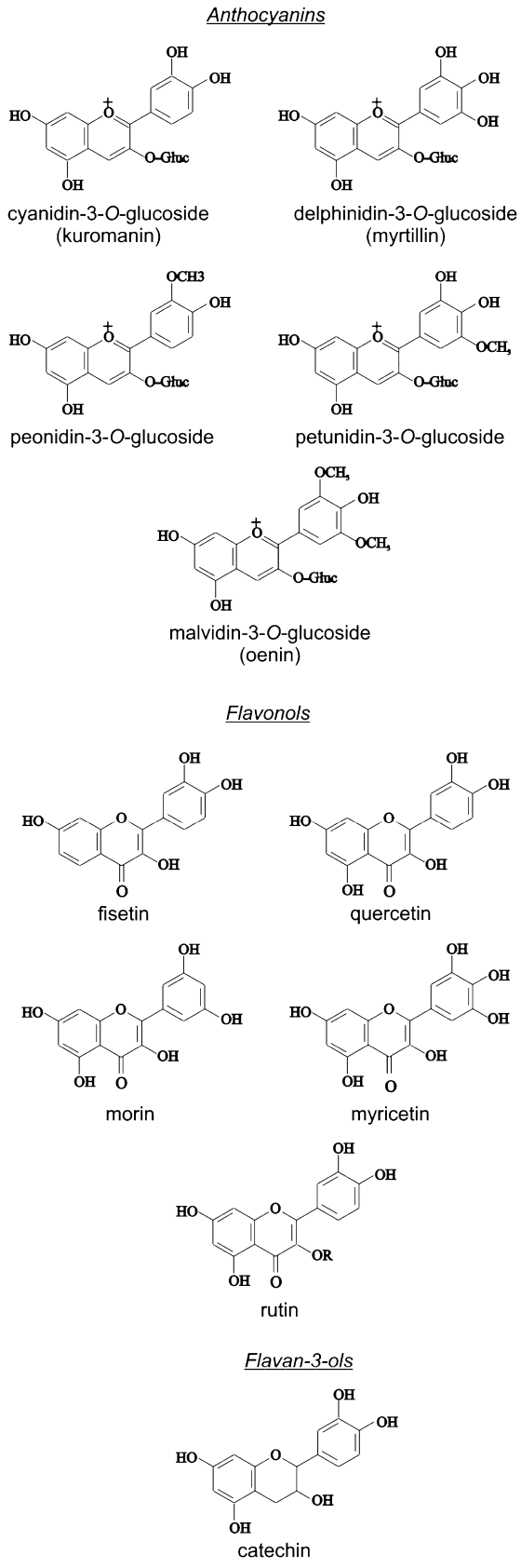

Fig. 2 – Structures of flavonoids.