Food Research International 69 (2015) 21–25

Contents lists available at ScienceDirect

Food Research International

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

Short communication

Do white grapes really exist?

Panagiotis Arapitsas [a,*], Joana Oliveira [b], Fulvio Mattivi [a]

[a] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38010 San Michele all´Adige, Italy
[b] Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal

ARTICLE INFO

Article history:
Received 11 August 2014
Accepted 3 December 2014
Available online 7 December 2014

Keywords:
Pigments
Vitis vinifera L
Vitisins
Metabolites
Viticulture
Wine
Anthocyanins

ABSTRACT

Anthocyanins are natural coloured pigments representing part of the protective mechanism of many plants, including *Vitis vinifera* L., and they have a considerable influence on wine quality. Moreover, the presence of anthocyanins in ripe berries is used as the accepted qualitative parameter for distinguishing red from white grapes, since these pigments are known to be present only in red berries. On the other hand, pyranoanthocyanins are important pigments for the colour stability of red wines and are known to be formed after berry crushing, during vinification and wine ageing.

In this work, for the first time we provide clear evidence that the skin of international white grape cultivars (Chardonnay, Sauvignon Blanc and Riesling) contains measurable traces of anthocyanins. In addition, for the first time we report clear proofs about the presence of pyranomalvidin 3-*O*-glucoside (B-type vitisin) and carboxypyranomalvidin 3-*O*-glucoside (A-type vitisin) in fresh grapes and we quantify them.

1. Introduction

Anthocyanins are natural colourants, representing the largest and the most well-known class of flavonoids and they play an important role in plant physiology. In *Vitis vinifera* L. grape berries, anthocyanins are found in the skin of red (as well as black and pink) grape cultivars, and their concentration depends essentially on environmental parameters, while their composition is more closely linked to genetic factors (Castellarin & Di Gaspero, 2007; Fournier-Level, Lacombe, Le Cunff, Boursiquot, & This, 2010; Fournier-Level et al., 2009; Pelsy, 2010). The grape synthesises anthocyanins for protection against abiotic stress, such as solar exposure and UV radiation, cold and drought, but also to attract seed dispersals. Moreover, the anthocyanin profile is of great importance from the taxonomical point of view, since it is relatively stable for each cultivar (Flamini, Mattivi, De Rosso, Arapitsas, & Bavaresco, 2013; Mattivi, Guzzon, Vrhovsek, Stefanini, & Velasco, 2006).

To our knowledge, anthocyanins are known to be present only in red grape cultivars, and in both analytical chemistry and biology this qualitative characteristic is used to define the difference between the two types of grape, red vs white (Castellarin & Di Gaspero, 2007; Flamini et al., 2013; Walker et al., 2007). The concentration and the pattern of the pigments in the berry skin can also explain the attribution of "pink" vs "red" *vs* "black" genotypes (Pelsy, 2010). Very recently, it was reported that the reason of the pink colour wine made from the white *V. vinifera* L. Síria grape cultivar was the presence of anthocyanins in the grape skin and pulp (Andrea-Silva et al., 2014).

Pyranoanthocyanins such as A and B-type vitisins (Fig. 1C) on the other hand, are pigments which are formed during the vinification and ageing of wine (Arapitsas, Perenzoni, Nicolini, & Mattivi, 2012; Arapitsas, Speri, Angeli, Perenzoni, & Mattivi, 2014; Arapitsas et al., 2012; De Freitas & Mateus, 2011). A-type vitisin (carboxypyranomalvidin 3-*O*-glucoside) is a product from the reaction between malvidin 3-*O*-glucoside and pyruvic acid, while B-type vitisin (pyranomalvidin 3-*O*-glucoside) is a product from the reaction between malvidin 3-glucoside and acetaldehyde (Bakker & Timberlake, 1997; Fulcrand, Dos Santos, Sarni-Manchado, Cheynier, & Favre-Bonvin, 1996). However, lately two works have demonstrated that pyranoanthocyanins in red grapes can be formed post-harvest, during the drying process (Marquez, Dueñas, Serratosa, & Merida, 2012, 2013).

The object of this study was to examine whether white grapes contain any measurable amounts of anthocyanins and whether pyranoanthocyanins are present generally in grapes. For this purpose a new and validated and systematic ULPC-MS/MS method was used, which was shown to be sensitive enough to also detect and quantify anthocyanins in trace amounts (Arapitsas, Perenzoni, Nicolini, & Mattivi, 2012; Ehrhardt, Arapitsas, Stefanini, Flick, & Mattivi, 2014; Sternad Lemut, Trost, Sivilotti, Arapitsas, & Vrhovsek, 2013).

2. Materials and methods

Methanol (HPLC for the extraction and LC-MS grade for the LC-MS analysis) was purchased from Fluka (Italy), and formic acid (LC-MS grade) was purchased from Sigma Aldrich (Italy). The 3-*O*-glucosides

* Corresponding author.
E-mail addresses: panagiotis.arapitsas@fmach.it, panagiotis.arapitsas@gmail.com (P. Arapitsas).

http://dx.doi.org/10.1016/j.foodres.2014.12.002
0963-9969/

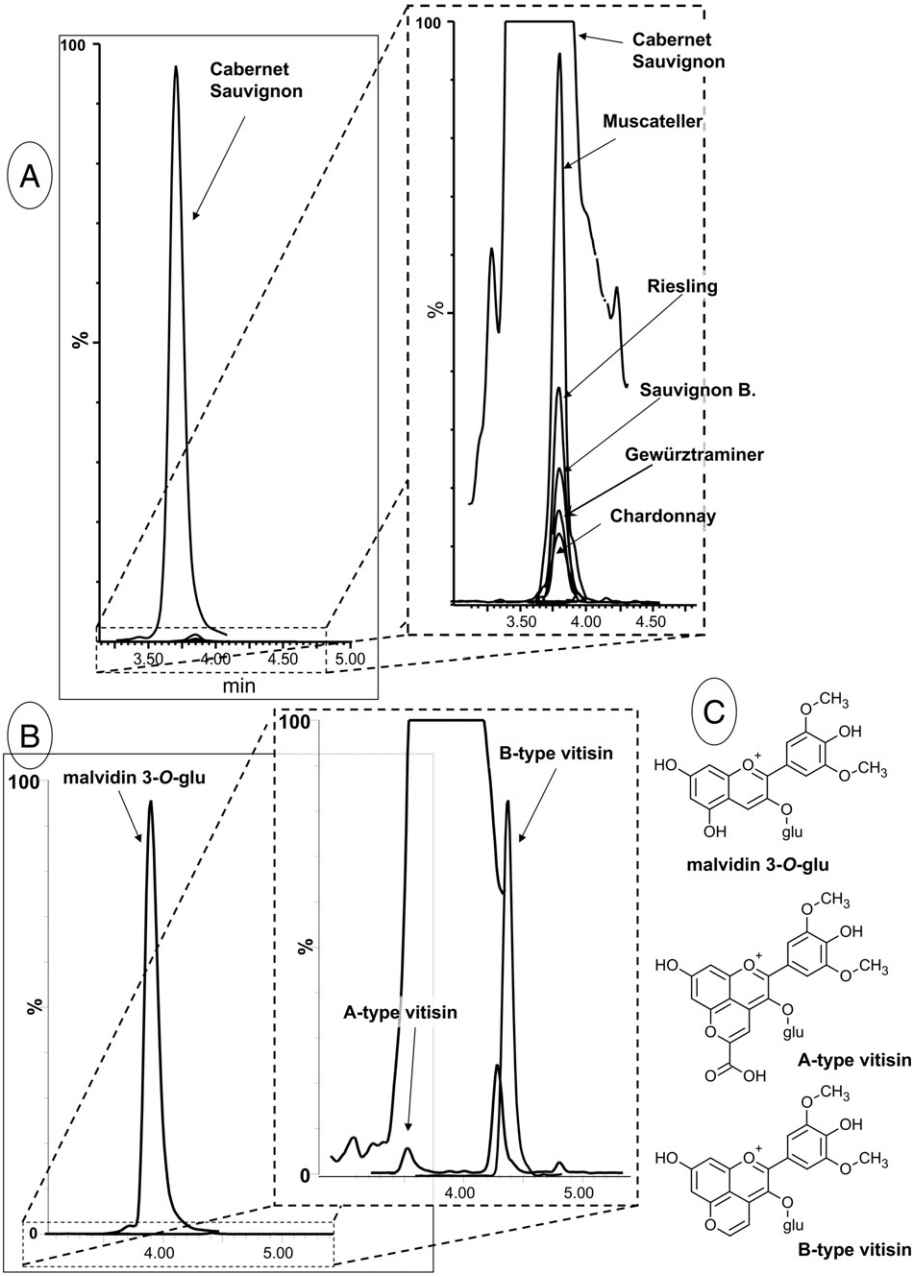

Fig. 1. (A) Comparison of the malvidin 3-*O*-glucoside LC-MS chromatographic peaks of the red Cabernet Sauvignon, the pink Gewurztraminer and Moscateller, and the white Sauvignon Blanc, Riesling and Chardonnay cultivar. (B) Comparison of the LC-MS chromatographic peak of malvidin 3-*O*-glucoside with A and B-type vitisin. (C) Structures of malvidin 3-*O*-glucoside, A and B-type vitisin.

and 3,5-*O*-diglucosides of malvidin, delphinidin, cyanidin, peonidin, petunidin and pelargonidin reference standards were of the highest purity grade available and purchased from Polyphenols Laboratories AS (Sandnes, Norway). The 3-(6′′-acetyl)-*O*-glucosides of peonidin and malvidin, and the 3-(6′′-*p*-coumaroyl)-*O*-glucosides malvidin, delphinidin, cyanidin, peonidin, and petunidin were isolated as previously reported (Arapitsas, Perenzoni, Nicolini, & Mattivi, 2012; Arapitsas, Scholz, et al., 2012). A and B-type vitisins were obtained as previously described (Oliveira, De Freitas, & Mateus, 2009; Oliveira et al., 2006). Milli-Q water was used for the chromatography and preparation of several standard solutions.

All *V. vinifera* L. grapes were harvested from the Fondazione Edmund Mach vineyards (San Michele all′Adige, Trentino, Italy) at technological maturity and then directly frozen and stored at −20 °C. In 2012 the red cultivars Sangiovese, Cabernet Sauvignon and Merlot, the pink cultivar Gewürztraminer, and the white cultivars Riesling and Sauvignon Blanc were sampled, while in 2011 the same cultivars were sampled, plus Chardonnay (white) and Moscateller (pink). For each sample ~500 g was harvested, and 20 berries were randomly taken from this initial cluster three times, in order to create three biological replicates for each sample.

The 20 berries of each biological replicate were weighed and peeled; and the skins were grounded in a mortar and transferred into a 15 mL amber vial. The vial was filled with methanol, shaken with an orbital shaker for 30 min at room temperature, centrifuged for 5 min at 4 °C and 5000 rpm and the liquid phase was transferred into a 50 mL flask. A second extraction then took place by refilling the vial up to 15 mL, shaking and centrifuging as before, and the liquid was transferred into the same flask, which was then filled up to 50 mL with Milli-Q-water. Sample preparation and analysis were occurred few days after the sampling. Finally, the extract was filtered through a 0.22 μm filter into a LC-MS certificated vial (Arapitsas, Perenzoni, Nicolini, & Mattivi, 2012; Arapitsas, Scholz, et al., 2012; Ehrhardt et al., 2014).