Study of grape bioactive stilbenes by suspect screening metabolomics

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Abstract. Stilbenes are one of the main classes of grape polyphenols associated with the beneficial effects of drinking wine. In the present study, a “suspect screening” metabolomics approach was used to study these grape compounds. Analysis was performed by Ultra High Performance Liquid Chromatography/Quadrupole Time-Of-Flight (UHPLC/QTOF) mass spectrometry and compounds were identified using the database GrapeMetabolomics expressly constructed. By this approach, a total of 18 stilbene derivatives was identified in two V. vinifera red grape varieties (cvs. Raboso Piave and Primitivo) on the basis of accurate mass measurements and isotopic patterns. Identifications were confirmed by multiple mass spectrometry (MS/MS). Bioactive stilbenes such as trans-resveratrol, Z- and E-piceid, picatannol and Z- and E-astringin, and several resveratrol dimers, trimers and tetramers such as pallidol and pallidol-3-O-glucoside, E- and Z-epi-viniferin, E- and Z-δ-viniferin, caraphenol B, parthenocissusin A, E- and Z-miyabenol C, hophepenol, ampelopsin H, vaticanol C-like or isohopeaphenol, were identified. In our knowledge, some of these resveratrol trimers and tetramers were reported in grape for the first time.

1. Introduction

Stilbenes are the grape compounds also present in the wine and are one of the main classes of polyphenols associated with the beneficial effects of drinking wine [2]. An epidemiological study carried out in the late 1970s showed that in France, despite the high consumption of food rich in saturated fatty acids, the incidence of mortality from cardiovascular diseases was lower than in other comparable countries. This phenomenon, called the “French paradox”, was correlated with the beneficial effects of consuming red wine as a major factor [17].

Resveratrol showed anti-cancer, anti-oxidant and anti-inflammatory activity, confers cardioprotection and inhibits platelet aggregation [3, 7, 8, 11, 12, 16].

Piceatannol was reported to block LMP2A (viral protein-tyrosine kinase implicated in leukemia, non-Hodgkin’s lymphoma and other diseases associated with the Epstein-Barr virus), and to act on human melanoma cells [9, 14, 19]. Viniferins and resveratrol trimers and tetramers are formed by oligomerization of trans-resveratrol in grape tissues as active defense against exogenous attack, or produced by extracellular enzymes released from pathogens in an attempt to eliminate undesirable toxic compounds [4, 18].

Metabolomics is the comprehensive qualitative and quantitative study of all the metabolites in a biological system (cell, tissue or organism). In general, ‘untargeted metabolomics’ provides high sensitivity, good resolution and high-throughput capacity, and in wines can reveal several thousand signals of candidate biomarkers in a single run [1]. Targeted metabolomics is performed for quantitative studies on specific compounds and most of the metabolome information of complex samples, such as wines, is missed [5, 21]. ‘Suspect screening analysis’ is a mid-way approach. This
method of identification of metabolites relies on the availability of specific information on compounds, e.g., their molecular formula and structure [13]. In the present study, suspect screening metabolomics was used to study stilbenes in two red grape varieties (Raboso Piave and Primitivo). Compounds were characterized by accurate mass spectrometry analysis using an Ultra High Performance Liquid Chromatography/Quadrupole Time-Of-Flight (UHPLC/QTOF) system and identifications were confirmed by multiple mass spectrometry (MS/MS) analysis.

2. Results

Raboso Piave and Primitivo grape samples were collected in 2011 at full ripeness from the CRA-VIT grapevine Germoplasm Collection (Susegana, Veneto, Italy). Berries were homogenized using liquid nitrogen and extracted with pure methanol. Identification of stilbenes was performed using the database GrapeMetabolomics expressly constructed with specific information available in the literature and other databases, such as their molecular formula and isotopic pattern [6]. Currently, this database contains around 1,000 putative metabolites of grape and wine. After a targeted search in GrapeMetabolomics, the raw data are processed by an untargeted algorithm and, if the search for resulting molecular formulae in other databases provides identification of a new compound, it is added to the database. As a consequence, by increasing the number of samples studied, GrapeMetabolomics can be expanded. Currently, more than 50 V. vinifera grape varieties included in the Vigneto Project have been studied. By this approach, performing two analyses of a grape extract (positive and negative ionization mode) the molecular formulae of 320–450 metabolites are detected including polyphenols, anthocyanins, stilbene derivatives, flavones, flavanols, procyanidins, stilbenes, phenolic acids, glycoside aroma precursors.

In Raboso Piave grape extract about 80 antioxidant polyphenolic compounds were identified with score higher 95%, among them 18 stilbene derivatives. Bioactive stilbenes identified in the two-grape samples were: trans-resveratrol, Z- and E-piceid, piceatannol and Z- and E-astringin, and several resveratrol dimers, trimers and tetramers such as pallidol and pallidol-3-O-glucoside, E- and Z-a-viniferin, B, caraphenol B, parthenocissin A, E- and Z-miyabenol C. Precursor ions at m/z 679.197 and the corresponding MS/MS fragments were assigned to trimers E- and Z-miyabenol C previously found in V. vinifera leaves, [15]), and (+)-viniferol D (reported in V. vinifera stems, [20]); the precursor ions at m/z 905.260 and their MS/MS fragments were assigned to tetramers hopheapenol (previously found in red wine, [10], and to amelopsin H, vaticanol C-like or isohopeaphenol (found in V. vinifera leaves, [15]).

In our knowledge, this is the first time that these resveratrol trimers and tetramers were found in grape. In general total content of stilbenes was 5 mg/Kg grape (for Raboso) and 10 mg/Kg grape (in Primitivo).

Comparison between two samples showed similar trans-resveratrol content (1.1 mg/Kg), but Primitivo grape had higher dimers, trimers and tetramers, glucoside stilbenes and piceatannol content.

To our knowledge, this is the first time that such detailed qualitative and quantitative profiling of grape stilbene derivatives was reported. Although GrapeMetabolomics is not an open-source database and is specific for the enological field, this approach potentially can be applied to the metabolomics of other plant varieties.

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References


