



Research article

Grapevine tissues and phenology differentially affect soluble carbohydrates determination by capillary electrophoresis



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ARTICLE INFO

Article history:

Received 12 May 2017

Received in revised form

7 July 2017

Accepted 8 July 2017

Available online 8 July 2017

Keywords:

Capillary electrophoresis

Matrix effect

Plant tissues

Sugars

Vitis vinifera L.

ABSTRACT

Soluble carbohydrates distribution depends on plant physiology and, among other important factors, determines fruit yield and quality. In plant biology, the analysis of sugars is useful for many purposes, including metabolic studies. Capillary electrophoresis (CE) proved to be a powerful green separation technique with minimal sample preparation, even in complex plant tissues, that can provide high-resolution efficiency. Matrix effect refers to alterations in the analytical response caused by components of a sample other than the analyte of interest. Thus, the assessment and reduction of the matrix factor is fundamental for metabolic studies in different matrices. The present study evaluated the source and levels of matrix effects in the determination of most abundant sugars in grapevine tissues (mature and young leaves, berries and roots) at two phenological growth stages. Sucrose was the sugar that showed the least matrix effects, while fructose was the most affected analyte. Based on plant tissues, young leaves presented the smaller matrix effects, irrespectively of the phenology. These changes may be attributed to considerable differences at chemical composition of grapevine tissues with plant development. Therefore, matrix effect should be an important concern for plant metabolomics.

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1. Introduction

The allocation of carbohydrates in different plant tissues determines the biomass accumulation, and hence crop yield and fruit quality (Calenge et al., 2006; Godt and Roitsch, 2006; Jammer et al., 2015; Roitsch, 1999; Zhu et al., 2007). Also, defines bud fertility since inflorescence induction and development depends on sugars availability (Bennett et al., 2005). The carbohydrates are produced in photosynthetic tissues, and are either stored or transported to different sinks. In grapevine, as in other higher plants, sucrose is the predominant metabolite for carbon transportation, and the partition of sugars is determined by the relative sink strength (Avidad, 1982; Lecourieux et al., 2014; Zapata et al., 2004). Hence, growing leaves, fruits, roots, and other storage organs compete for the photoassimilates (Albacete et al., 2011; Biemelt and Sonnewald, 2006; Xiang et al., 2011; Yang et al., 2004). Considering that the

quality of wine is correlated with grape berry metabolic profile, an adequate sugar accumulation in the fruits is desired not only for ethanol production via fermentation, but also for the biosynthesis of compounds related to flavor (Hornsey, 2007). Sugars, especially glucose and fructose, are responsible for the sweet taste of grape juice and wine, and indirectly for ethanol and glycerol wine levels (Hufnagel and Hofmann, 2008).

Carbohydrate analysis is required for a variety of purposes such as food and beverage analysis, but also for the evaluation of plant physiology and metabolic studies. Gas chromatography coupled to flame ionization detection or mass spectrometry are common techniques for sugar analysis; however, multi-step derivatization is required for the sugars to become compatible with these chromatographic modes (Moreno et al., 2011; Murcia et al., 2016). Thus, several analytical approaches have been proposed for their direct determination in plant matrices. Commonly used techniques for underivatized carbohydrates are high performance liquid chromatography (HPLC) with pulsed amperometric detection, refractive index detector or evaporative light scattering detector (Carballo et al., 2014; Cataldi et al., 2000; Eyéghé-Bickong et al., 2012; Ma et al., 2014; Rocklin and Pohl, 1983; Soga et al., 1992). Nevertheless, HPLC is not optimally suited for routine analysis and sample

Abbreviations: BGE, background electrolyte; CE, capillary electrophoresis; HPLC, high performance liquid chromatography.

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<http://dx.doi.org/10.1016/j.plaphy.2017.07.010>

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pre-treatment is necessary to remove interfering compounds present in complex plant matrices (Oliver et al., 2014; Weitzhändler et al., 1996). Besides, chromatographic methods involve long analysis times, mainly due to lengthy column re-equilibration following the analysis of samples with complex matrices.

Capillary electrophoresis (CE) is a powerful separation technique that can provide high-resolution efficiency with minimal sample preparation. Due to the low cost per analysis, robustness and versatility, CE is becoming a standard tool for the analysis of agricultural interest compounds, even if complex matrices are involved (Landers, 2007). Nevertheless, since carbohydrates lack both a charge and a strong UV chromophore, most of CE approaches involve the complexity of derivatization, a time-consuming step (Guttman, 1997; Guttman et al., 1996; Honda et al., 1989, 1991; Suzuki et al., 2003). Alternatively, CE methods for the analysis of underivatized carbohydrates have been developed. These methods include the use of high alkaline electrolyte to ionize the carbohydrates and they are suitable for indirect UV detection (Cabálková et al., 2004; Jiang et al., 2015; Klockow et al., 1994; Rizelio et al., 2012; Soga and Serwe, 2000; Vorndran et al., 1992). The key advantages of CE over HPLC are that undesirable sample components can be easily and quickly flushed out after analysis. Moreover, a new capillary is much more affordable than a new HPLC column.

It has to be pointed out that matrix effect can be a serious problem in CE analysis of sugars, affecting quantitative analysis and method reproducibility (Piñero et al., 2011). Significant differences in the analytical signals are obtained between standard solutions and doped-samples as the result of chemical and/or physical interactions of sugars and matrix components with the capillary wall (Piñero et al., 2011). Little is known about matrix effect in the determination of soluble carbohydrates by CE with indirect detection. Indeed, there are no evidences concerning its relation with different plant tissues and/or development stages. Thus, the main purpose of the present work is to evaluate the source and levels of matrix effects in the determination of soluble sugars in different plant matrices. The optimized methodology was successfully applied for the determination of glucose, fructose and sucrose in young and mature leaves, berries and roots of a grapevine at the onset of ripening (pre-veraison) and veraison.

2. Material and methods

2.1. Plant material and sugar extraction

One-year-old grapevines of a selected clone of *Vitis vinifera* L. cv. Malbec were cultivated in plastic pots under field conditions (33°0' S, 68°52' W, 940 m asl). The vines were shoot-thinned to one shoot per vine, and one cluster was left at flowering. Samples of different tissues were taken at two stages of growth and development, the pre-veraison (stage 35) and veraison (stage 36) (Coombe, 1995). Young leaves (fifth fully expanded leaf from the shoot apex), mature leaves (third leaf from the base of the shoot), berries, and roots were sampled (n = 5).

The procedure for sugar extraction was performed following previous studies (Moreno et al., 2011). Briefly, the extraction was done homogenizing 100 mg of tissue samples with 5 mL of 80:20 (v/v) ethanol:water using a disperser (Ultra-Turrax, T 10 basic; IKA, Staufen, Germany). Then, the mixture was left for 90 min at 70 °C, centrifuged for 10 min at 15,000 g, and supernatants were collected.

2.2. Standards and solutions

Glucose and fructose, both ≥95% (Sigma-Aldrich, Milwaukee, WI, USA), and sucrose ≥97.0% (Fluka, Buchs, Switzerland), were

used as standards for soluble sugars. Standard solutions containing the three sugars at concentrations between 80 and 500 mg L⁻¹ were prepared.

The background electrolyte (BGE) solutions were based on Sugar Analysis Chemical Kit, Beverages, version 20.01.2014 (Lumex Ltd, St Petersburg, Russia; www.lumex.ru). In addition, methanol and acetonitrile (HPLC grade purity, JT Baker, Deventer, Holland) were used. Hexadecyltrimethylammonium bromide (CTAB), hydrochloric acid, potassium sorbate, sodium hydroxide were purchased from Sigma-Aldrich. Solutions were prepared using ultrapure water (18.3 Mm) from a Milli-Q system (Millipore, Paris, France), and stored in amber-colored glass bottles at 4 °C.

2.3. Capillary electrophoresis (CE)

Analyses of underivatized carbohydrates in different grapevine extracts were performed with reverse polarity and dynamic coating using a Capel 105M (Lumex Ltd, St Petersburg, Russia) system equipped with an UV detector and a 0–25 kV high-voltage power supply. Data were collected on a PC configured with Elforun software version 3.2.2. Capillary columns were bare fused-silica capillaries 55 cm effective length with 75 µm ID and 375 µm OD (MOLEX Incorporated, Polymicro Technologies, Phoenix, AZ, USA). Sample supernatants and standard solutions were filtered through 0.45 µm membrane pore size and centrifuged at 10,000 g for 5 min, just before being introduced into the capillary by pressure injection at 30 mbar for 5 s. Electropherograms were performed at 254 nm with Indirect UV detection, and all operations were carried out at 20 °C. Then, sugars were determined according to the protocol from Sugar Analysis Chemical Kit with modifications.

2.4. Evaluation of matrix effect

A matrix effect is defined as a change in the analytical signal caused by anything else in the sample other than analyte. The most effective way to evaluate matrix effect affecting trueness and precision of the analytical method is to use the standard addition technique (Stüber and Reemtsma, 2004). Standard addition is especially appropriate when the sample composition is unknown or complex and affects the analytical signal. If small volume of concentrated standard is added, the concentration of the matrix will not be significantly changed (Ćirić et al., 2012).

The matrix effect during the development of the analytical method was examined by comparing the analytical response (peak areas) of an analyte in spiked grapevine tissue extracts with the response of the same analyte present in pure solvent at several concentration levels.

The relative matrix effect was calculated following equation (1), where X1 and X2 are the slopes of each analyte calibration curves in the samples and in pure solvent, respectively (Gomez et al., 2013).

$$\text{Matrix effect (\%)} = 100 - [(X1/X2) \times 100] \quad (1)$$

3. Results and discussion

3.1. CE separation

The Lumex protocol for sugar analysis (Sugar Analysis Chemical Kit, Beverages, version 20.01.2014, Lumex Ltd, St Petersburg, Russia) was developed for alcoholic drinks and we found that reproducibility for such approach was not adequate for our plant extracts. Therefore, organic modifiers such as acetonitrile and methanol (2–20% v/v) were used to enhance the reproducibility and resolution. Additionally, we tested the experimental conditions for the

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