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Characterization of anthocyanins in novel Chilean maqui berry clones by HPLC–DAD–ESI/MS^n and NMR-spectroscopy

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ABSTRACT

Anthocyanins in two Chilean maqui berry (*Aristotelia chilensis* (Mol.) Stuntz) clones named Luna Nueva and Morena were isolated and characterized by a core-shell column-based HPLC–DAD–ESI/MS^n method and 2D–NMR spectroscopy, focusing on the yet not fully elucidated glycosylation pattern of the aglycones. For the first time, 2D–NMR spectroscopic data unambiguously showed that the major maqui anthocyanins were delphinidin 3-*O*-(2″-*O*-*β*-xylopyranosyl-*β*-glucopyranoside)-5-*O*-*β*-glucopyranoside and delphinidin 3-*O*-*β*-glucopyranoside-5-*O*-*β*-glucopyranoside. Among these pigments, the latter represented the most abundant anthocyanin in Luna Nueva (59% of total anthocyanin content, TAC) and Morena (50% of TAC). TAC was significantly higher in Luna Nueva (14.6 g/kg dry weight, DW) than in Morena (12.8 g/kg DW). In both samples, relative proportions of diglycosylated anthocyanins (84% of TAC) exceeded those of mono-substituted anthocyanins (~16%). The provided information about the position of glycosylation may be relevant for assessing color stability of maqui anthocyanins in future food applications.

1. Introduction

In recent years, maqui berry (*Aristotelia chilensis* (Mol.) Stuntz, Elaeocarpaceae) has gained increasing scientific and economic interest due to its outstandingly high anthocyanin content. Maqui is an evergreen shrub or small tree with a height of up to 5 m. It is believed to originate from an area ranging from the Andean region of Argentina to the temperate forests of Central and South Chile (Fredes et al., 2012; Vogel et al., 2014). In these regions, in December and January, maqui produces intensely violet to blackish colored berries of about 5 mm in diameter containing one to eight angular seeds (Rodríguez, 2005). The edible berry has been used to produce juice, pulp, jam, and liqueur as well as, due to its high tinctorial strength, to enhance color of pale red wines (Benn Brothers, 1890; Royal Botanic Gardens, 1890). The strikingly high anthocyanin level in maqui berry is not only responsible for its coloring properties, but also for its exceptionally high antioxidant activity, being associated with potential health benefits such as anti-inflammatory, anti-diabetic, and cardioprotective effects (Davinelli et al., 2015; Fredes et al., 2012; Gironés-Vilaplana et al., 2014; Miranda-Rottmann et al., 2002; Rojo et al., 2012; Schreckinger et al., 2010; Vergara et al., 2015). In addition to anthocyanins, maqui berry has been recently reported to contain further health-promoting compounds, micronutrients and minerals, in particular, unsaturated fatty acids in its seeds (Brauch et al., 2016).

Previously, eight individual maqui anthocyanins have been characterized by HPLC–DAD–ESI/MS^n, representing putative 3-*O*-sambubioside-5-*O*-glucosides and 3-*O*-glucoside-5-*O*-glucosides as well as clearly identified 3-*O*-sambubiosides and 3-*O*-glucosides of delphinidin and cyanidin (Brauch et al., 2016; Céspedes et al., 2010; Escribano-Bailón et al., 2006; Genskowsky et al., 2016; Girones-Vilaplana et al., 2012; Gironés-Vilaplana et al., 2014; Ruiz et al., 2010; Schreckinger et al., 2010). The structural elucidation of 3-*O*-mono-substituted maqui anthocyanins was confirmed by using authentic standards. However, the glycosylation positions of

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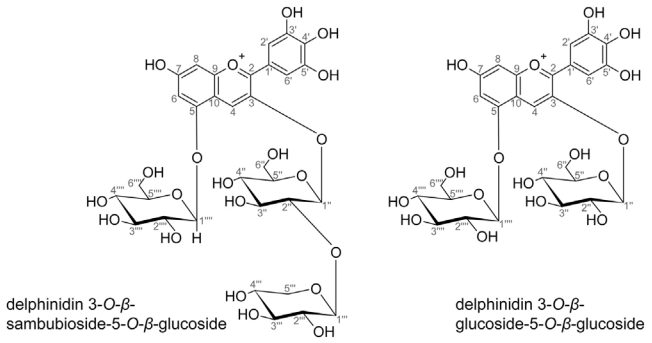

Fig. 1. Chemical structures of delphinidin 3-*O*-sambubioside-5-*O*-glucoside and delphinidin 3-*O*-glucoside-5-*O*-glucoside.

the putative 3,5-*O*-diglycosylated maqui anthocyanins (Fig. 1) remained under debate, as their UV/Vis absorption and mass spectral properties might be ambiguous, e.g. when comparing to 3,7-*O*-diglycosylated anthocyanins. For instance, a rather unusual 3,7-*O*-diglucoside substitution has been recently characterized in calafate (*Berberis microphylla* G. Forst) berry by NMR spectroscopy (Ruiz et al., 2014). Therefore, studies implementing authentic reference compounds or 2D–NMR spectroscopy are needed for the correct assignment of the position of the glycosyl moieties. The relative proportions of individual anthocyanins and their total content may be of great interest when evaluating their color stability in future food applications. For instance, stabilities of 3-*O*-mono- and 3,5-*O*-diglycosylated anthocyanins have been reported to differ in aqueous solutions with varying pH values (Brauch et al., 2015).

Thus, the objective of the presented study was to elucidate the glycosylation pattern of maqui anthocyanins by HPLC–DAD–ESI/MS^n and 2D–NMR spectroscopy. In addition, we will briefly report about two novel maqui clones ("Luna Nueva" and "Morena") that have been obtained by selection of early-fruiting and high-yielding clones out of a large germplasm collection cultivated in experimental designs at five Chilean locations (Vogel et al., 2014). The availability of such clones being currently restricted to Chile might be of particular interest for commercial purposes due to the increasing demand for maqui, particularly for dried berries having extended shelf life suitable for exportation. To date, the berries of Luna Nueva and Morena are only available in their dried form outside of South America to preserve them for transport and storage.

2. Materials and methods

2.1. Chemicals and plant material

Authentic standards of the 3-*O*-glucosides of cyanidin (≥96%) and delphinidin (≥95%) were purchased from Extrasynthèse (Genay Cedex, France). All further chemicals used were supplied by Merck and VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Ultrapure water was produced with a Milli-Q purification system (EMD Millipore, Bedford, MA, USA) and used for all experiments.

Fresh and dried maqui berries were obtained from selected clones that had been produced by the following breeding process. In 2007, fruit samples were taken from the 30 highest yielding explants from each of nine wild populations of maqui with more than 50 plants per population located in Central and South Chile (latitudes between 34° and 41° S). In each of the wild populations, the ten explants with highest total monomeric anthocyanin contents, as determined by pH-differential method according to Giusti and Wrolstad (2004), were selected. After vegetative propagation, a total of 68 clones was planted in the experimental station of the University of Talca (Talca, Chile) in 2009. Three years later, 45 of the earliest fruit producing clones were further investigated in a randomized experimental design with five replicates in five Chilean locations (latitudes between 35°–40° S). Among these, the clones named Luna Nueva and Morena (see photograph in Fig. 2) were selected for the present study due to their high fruit production during the 2nd and 3rd seasons. The fruit of these clones were manually harvested in 2014 from three-year-old shrubs cultivated in the experimental station and stored at −20 °C until further use. Aliquots of the collected samples were dried at 70 °C for 24 h in a hot air circulation oven (Memmert ULM 700, Schwabach, Germany), packed into paper bags and shipped to the Institute of Food Science and Biotechnology (University of Hohenheim, Stuttgart, Germany) on the same day, where they were stored at −80 °C. For subsequent anthocyanin extraction, 450–500 dried berries (ca. 50 g) were used. Fruit samples were dried to avoid quality loss by decay during the shipment from Chile to Germany. The difference in weight between fresh and dried samples (humidity loss [%]) amounted to 43 and 48% for Luna Nueva and Morena, respectively. Final moisture contents of the dried fruit samples as determined by infrared drying at 85 °C (Infrared Moisture Analyzer, Sartorius, Göttingen, Germany)

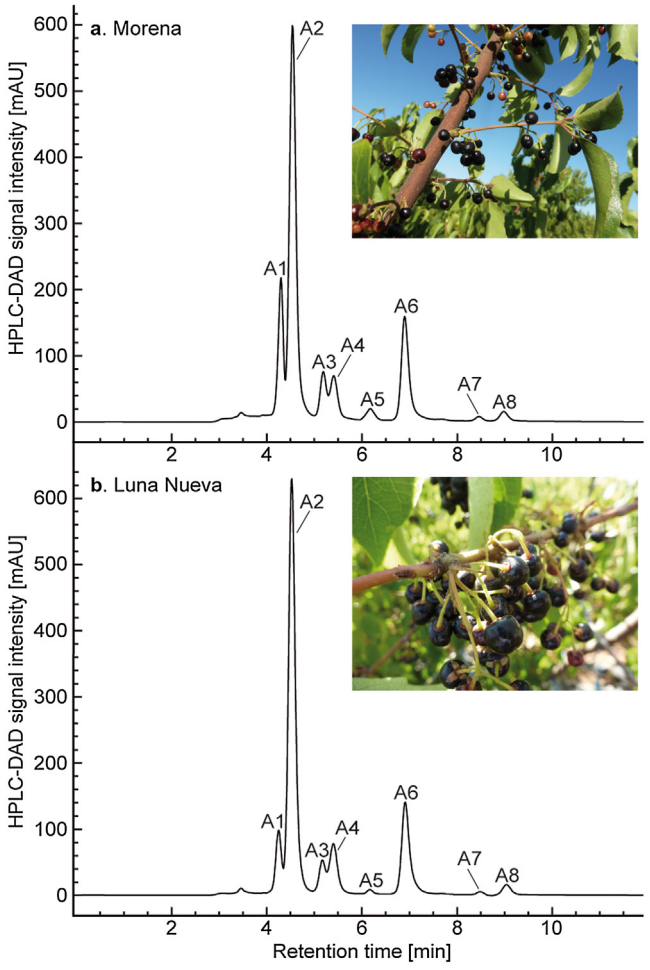

Fig. 2. HPLC separation of maqui berry anthocyanins in the dried berry of Morena (a) and Luna Nueva (b) monitored at 520 nm. For peak assignment, see Table 1.