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Effects of latitude and weather conditions on proanthocyanidins in berries of Finnish wild and cultivated sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*)

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ABSTRACT

Sea buckthorn (*Hippophaë rhamnoides* ssp. *rhamnoides*) of varieties 'Terhi' and 'Tytti' and one of wild origin were cultivated in southern and northern Finland, harvested during 2007–2013. Proanthocyanidins (PAs) were analyzed with HILIC UPLC–ESI-MS. The southern and northern samples were separated in the partial least squares discriminant analysis model (four factors, R^2 0.75, Q^2 0.70). The total PAs were more abundant in berries from the north (610–970 mg/100 g DW) than in those from the south (340–450 mg/100 g DW) ($p < 0.05$). In northern Finland, the length of the growth season as well as the temperature sum and radiation sum of the growth season until harvest were negatively correlated with the total PAs in all the samples but positively with PA oligomers in 'Tytti' and 'Terhi'. In southern Finland no respective correlations were seen. 'Terhi' and 'Tytti' had different trends in the content of total PA and oligomers in overripe stages.

1. Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) is a deciduous species of the family Eleagnaceae native to Eurasia. As a hardy (temperature, salt and drought resistant) ornamental shrub or tree, the plant has attracted a great deal of attention in many countries. It has economic potential and environmental value in e.g. soil improvement and sand fixation (Dhyani, Maikhuri, & Dhyani, 2011). In the past decade, numerous studies have shown that sea buckthorn berries and products are associated with multiple health benefits (Joseph, Edirisinghe, & Burton-Freeman, 2014; Lehtonen et al., 2011; Xu, Kaur, Dhillon, Tappia, & Dhalla, 2011; Yang & Kortesniemi, 2015). A recent study suggested that the berries might serve as a new prebiotic source for functional foods (Gunenc et al., 2016).

Proanthocyanidins (PAs) are a group of polyphenolic secondary metabolites. Oligomeric and polymeric PAs, which are composed of flavan-3-ol units linked by C4-C8 and/or C4-C6 bonds, are categorized as B-type PAs. A-type proanthocyanidins contain additionally C2-O-C7 ether bonds between the monomer units (Ou & Gu, 2014). The complex structures and varying composition endows the PAs with multiple biological and biochemical properties. The compounds are present in many organs and tissues of plants to increase disease resistance, to reinforce plant tissues and to restrict the growth of neighbouring plants (Bais, Vepachedu, Gilroy, Callaway, & Vivanco, 2003; Lepiniec et al., 2006; Santos-Buelga & Scalbert, 2000). These properties are not only reflected in the protection of plants, but also in the potential biological activity on human health (Hsieh, Shen, Kuo, & Hwang, 2008; Rasmussen, Frederiksen, Struntze Krogholm, & Poulsen, 2005; Terra et al., 2011). Moreover, PAs are present in many fruits and plant products especially in wine, fruit juices and tea contributing to astringency and bitter taste of food (Laaksonen, Salminen, Mäkilä, Kallio, & Yang, 2015; Peleg, Gacon, Schlich, & Noble, 1999; Sarni-Manchado, Cheynier, & Moutounet, 1999).

The biosynthesis of PAs share common steps in the phenylpropanoid and flavonoid pathway and the MYB (Myeloblast) family transcription factors are considered as the common regulators of PA biosynthesis in different plant tissues (He, Pan, Shi, & Duan,

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2008; Zhao, Pang, & Dixon, 2010). Genetic background of the plant is the main determinant of the content of PAs in plant tissues, but environmental factors may also affect the composition (Jaakola & Hohtola, 2010; Zoratti, Karppinen, Escobar, Häggman, & Jaakola, 2014). In a previous study, Carvalhoa et al. found that a long day photoperiod increased the content of catechins and flavonols in sweet potato leaf (Carvalho, Cavaco, Carvalho, & Duque, 2010). Light-inducible R2R3 MYB transcription factor was adjusted to regulate the biosynthesis of PAs in apple and grapevine (Gesell, Yoshida, Tran, & Constabel, 2014; Koyama, Ikeda, Poudel, & Goto-Yamamoto, 2012). Through the expression of a maize *bHLH* anthocyanidin regulatory gene (*Lc*), leaf tissue of alfalfa accumulated more PAs under stress conditions of high light intensity or low temperature (Wang, Frutos, Gruber, Ray, & McAllister, 2006). Hagen et al. showed that post-harvest light treatment increased the content of procyanidins in apple peel (Hagen et al., 2007). PAs in juniper needles and in sea buckthorn of varieties of *H. rhamnoides* ssp. *mongolica* were increased in northern Finland compared with those grown in southern Finland (Martz et al., 2009; Yang, Laaksonen, Kallio, & Yang, 2016).

Accumulation of PAs in plants is a result of the complex action influenced by various factors but the number of detailed long-term studies on the effect of environmental factors on PAs, are still limited. For these reasons, it is essential to investigate how environmental conditions such as latitude and climate influence the content and composition of PAs.

In our previous study, we compared the PAs in sea buckthorn of different subspecies and origins based on berries harvested in one year (Yang et al., 2016). In the present study, PAs were analyzed in berries collected for 3–7 years from wild and cultivated sea buckthorn grown in southern and northern Finland. The effects of growth latitude and various weather variables associated with latitude on accumulation of PAs were investigated using multivariate analysis models. The aim was to provide a more thorough knowledge of the effects of growth environment on PAs in sea buckthorn and to provide guidance for plant breeding and cultivation as well as for raw material selection for berry processing industry.

2. Materials and methods

2.1. Plant materials

Two Finnish varieties of sea buckthorn, 'Terhi' and 'Tytti' of *Hippophaë rhamnoides* ssp. *rhamnoides* were cultivated in Turku (22°09′ E, 60°23′ N, altitude 1 m) in southern Finland and in Kittilä (24°37′ E, 68°02′ N, altitude 210 m) in northern Finland. Wild ssp. *rhamnoides* berries were grown in Uusikaupunki (21°15′ E, 60°54′ N, altitude 1 m) in southern Finland and, in addition, bushes from the same growth place were transplanted in Kolari (24°42′ E, 67°07′ N, altitude 163 m) in northern Finland. Berries were harvested annually during 2007–2013 when optimally ripe as determined by local sea buckthorn experts (Supplementary Table 1). The berry collection time was typically from late August to early September in Turku and typically one month later in Kittilä. In addition, a series of samples were collected at different time points after ripening during September – mid December, 2011 from the two varieties 'Terhi' and 'Tytti' in Turku in order to study the post-ripening changes in PAs in sea buckthorn berries. The detailed collecting dates are presented in Supplementary Table 1.

The growth places had two to four field blocks each. Berries were harvested randomly from the bushes within each block, pooled and mixed well for each sample. All the samples were frozen immediately after picking and stored at −18 °C until analysis.

2.2. Sample preparation and purification

Extraction and purification of PAs was accomplished according to our previous methods (Yang et al., 2016). Briefly, about 10 g of sea buckthorn berries was weighed accurately in duplicate, thawed, crushed and extracted with a mixture of acetone, water and acetic acid (80:19.5:0.5, v/v). The extracts were evaporated to remove acetone. The remaining aqueous solution was defatted using Sephadex LH-20 (Pharmacia, Uppsala, Sweden) column chromatography. The column was eluted with 150 mL of water, 100 mL of methanol in water (20:80, v/v), 150 mL of acetone in water (70:30, v/v) and 100 mL methanol in sequence. The fraction of acetone in water was collected and re-dissolved in 1 mL methanol for analyses.

2.3. Proanthocyanidin analysis

The analysis was performed on Waters Acquity Ultra High Performance LC system (Waters Corp., Milford, MA), which consisted of a sample manager, binary solvent delivery system and Waters 2996 PDA Detector. In addition, Waters Quattro Premier Tandem Quadrupole mass spectrometer (Waters Corp., Milford, MA) equipped with an electrospray-ionization (ESI) source was combined to the system. The system was operated using MassLynx 4.1 software. PAs were separated on a Phenomenex Luna HILIC 200A column (3 μm, 150 × 3.00 mm, Torrance, CA) according to the degree of polymerization. Acetonitrile and formic acid/water (0.5:99.5, v/v) were used as solvents with gradient elution. The gradient elution and ESI-MS operating parameters were the same as in our previous report (Yang et al., 2016). Quantitative analysis of oligomers (dimers, trimers and tetramers) was carried out using HILIC-ESI-SIR, and total PAs with BL-DMAC assay as described previously (Yang et al., 2016). An external standard method was used for quantification. Oligomeric PAs were quantified with the calibration curves constructed by analyzing standard solutions of procyanidin B2 (Extrasynthese, Genay, France) in methanol in the concentration range of 0.01–10 mg/100 mL. For total PAs the calibration curves were constructed by reaction of DMAC with procyanidin B2 in acidified ethanol at final concentrations of 100–2000 mg/100 mL. The contents of dimeric, trimeric, tetrameric and total PAs were calculated by the aid of calibration curves and use of correction factors (Kallio, Yang, Liu, & Yang, 2014). Content of PAs in each sample was expressed as mg/100 mL in dried fruits.

2.4. Weather conditions

The meteorological data were provided by the Finnish Meteorological Institute (Helsinki, Finland). Data recorded at the weather stations of Kaarina Yltöinen (near Turku) (60°23′N, 22°33′E, 6 m) during 2007–2008, Turku Artukainen (60°27′ N, 22°10′ E, 8 m) during 2009–2013 and Kittilä Pokka (68°10′ N, 25°47′ E, 275 m) during 2007–2013 were used in this study. The weather variables and their abbreviations were shown in Supplementary Table 2.

2.5. Statistical analysis

All the samples were analyzed in duplicate. Statistical analyses and multivariate models were performed using SPSS 16.0.1 (SPSS Inc., Chicago, IL) and Unscrambler X, version 10.3 (CAMO Software, Oslo, Norway). A one-way analysis of variance (ANOVA) and independent-sample *t* test were performed to compare the differences in the composition between samples of different varieties, latitude and harvest time points. Partial least squares regression discrimination analysis (PLS-DA) was used to explain the difference among three varieties in the berry samples ($n = 36 \times 2$) according to the PA contents (X-data; $n = 16$), and the difference