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NMR metabolomics demonstrates phenotypic plasticity of sea buckthorn (*Hippophaë rhamnoides*) berries with respect to growth conditions in Finland and Canada

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ABSTRACT

The berries of sea buckthorn (*Hippophaë rhamnoides* ssp. *rhamnoides*) cultivars 'Terhi' and 'Tytti' were studied with respect to their growth location, 60° and 68° N latitude in Finland and 46° N in Canada, using ^{1}H NMR metabolomics. The berries of 'Terhi' were characterised by stronger signals of quinic acid, while 'Tytti' had higher levels of *O*-ethyl *β*-D-glucopyranoside. The metabolic profile of the northernmost berries was distinctly different from those grown in southern Finland or Canada. Berries from northern Finland had relatively higher levels of quinic acid, glucose, L-quebrachitol and ascorbic acid. Ethyl glucoside was shown to accumulate by several fold at the late stage of maturation in the south as it correlated with degree days ($r = 0.63$) and global radiation ($r = 0.59$), but not in the north. The variance in the composition of the sea buckthorn berries demonstrates plasticity in the acclimatisation to growth environments.

1. Introduction

Sea buckthorn (*Hippophaë* sp.) is a highly valued plant, in Europe and Asia, due to its nutritional, phytotherapeutic and environmental value (Bal, Meda, Naik, & Satya, 2011; Li & Schroeder, 1996). Leaves and berries and their fractions – fruit flesh, skin and seeds – are all rich sources of bioactive components (Bal et al., 2011; Fatima et al., 2015; Gutzeit, Baleanu, Winterhalter, & Jerz, 2008; Heinäaho, Hagerman, & Julkunen-Tiitto, 2009; Kallio, Yang, & Peippo, 2002; Ma et al., 2016; Zheng, Yang, Trépanier, & Kallio, 2012). These compounds may promote human health mainly through antioxidative and anti-inflammatory activity (Buchanan, Gruissem, & Jones, 2015; Ercisli, Orhan, Ozdemir, & Sengul, 2007; Gao, Ohlander, Jeppsson, Björk, & Trajkovski, 2000; Korekar, Dolkar, Singh, Srivastava, & Stobdan, 2014; Larmo, Yang, Hyssälä, Kallio, & Erkkola, 2014; Olas, Kontek, Malinowska, Żuchowski, & Stochmal, 2016; Xing et al., 2002; Xue et al., 2015; Yang & Kortesniemi, 2015).

Sea buckthorn is highly adaptable to biotic and abiotic stress factors. Studies abound focusing on the effect of geographical origin (especially latitude and altitude), genotype and maturation on the primary and secondary metabolism of sea buckthorn berries (Gao et al., 2000; Kallio et al., 2002; Kortesniemi, Sinkkonen, Yang, & Kallio, 2014; Li, Ruan, Silva, Guo, & Zhao, 2012; Su et al., 2014; Yang, Laaksonen, Kallio, & Yang, 2016; Zheng, Kallio, Linderborg, & Yang, 2011; Zheng et al., 2012). In particular, the effects of northern latitudes on the berry composition are of special interest. The Fennoscandia is one of the few locations where the "Nordic effect" on plants can be studied: wide areas at the same latitudes east- and westward are typically tundra and/or glaciers. Latitude of a location in the far north greatly affects the quantity and quality of solar radiation. Contrary to phytotron and controlled growth chamber experiments, the influence of ambient conditions can be studied as is in the natural growth environment. The northern conditions have been shown to produce berries with a higher content of bioactive compounds, such as flavonol glycosides and proanthocyanidins (Ma et al., 2016; Yang et al., 2016).

The Finnish sea buckthorn genotypes (*H. rhamnoides* ssp. *rhamnoides*) are generally characterised by relatively low levels of sugar and vitamin C, and high levels of total acid in the berries compared

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to other subspecies (Bal et al., 2011; Tang & Tigerstedt, 2001; Yang, 2009). After their release for cultivation in 2000, the female cultivars 'Terhi' and 'Tytti', originating from Finnish wild strains of ssp. *rhamnoides*, have become the main sea buckthorn cultivars in Finland. The cultivars were bred by MTT Horticulture, Finland. Winter-hardiness, resistance to disease (e.g. stem canker), moderate growth, and relatively high levels of vitamin C are their characteristic attributes. (Karhu, 2003.) Sea buckthorn is not native to Canada, but was initially introduced there as shelter trees in the early 20th century. Lately, due to the pharmaceutical and nutritional prospects, its cultivation has spread from Saskatchewan to Ontario (Fatima et al., 2015; Li & Beveridge, 2003). The quality of sea buckthorn berry can vary greatly between subspecies, cultivars, growth sites and harvesting time, and is influenced by post-harvest conditions (Gutzeit et al., 2008; Kallio et al., 2002; Kortesniemi et al., 2014; Li, Ruan, da Silva, Guo, & Zhao, 2012; Raffo, Paoletti, & Antonelli, 2004; Tang & Tigestedt, 2001; Xu, Hao, Yuan, & Gao, 2015; Yang et al., 2016; Zheng et al., 2012). Sugars, acids, vitamin C, carotenoids and phenolic compounds are key components affecting the quality of sea buckthorn berries (Ma et al., 2016; Raffo et al., 2004; Tiitinen, Hakala, & Kallio, 2005). Recently, the expression of the genes related to flavonoid, vitamin C, tocopherol, and lipid biosynthesis in sea buckthorn has been examined (Fatima et al., 2012; Fatima et al., 2015; Vuorinen et al., 2015).

The growth properties, composition, and nutritional value of sea buckthorn berries are affected by short growth period, long days during summer, low solar orbit, low total solar radiation, and long and harsh winters in the north. However, the long summer days in the northern latitudes near the Arctic Circle do not compensate for the low total irradiance, but serve as an extra stressor. The solar irradiance at sea level depends on the solar angle and the atmosphere to be passed. The phenotypic plasticity of the berries is especially of great importance with respect to the northern latitudes. For example, high latitude can increase the content of quinic acid and L-quebrachitol in ssp. *sinensis* (Kortesniemi et al., 2014; Zheng et al., 2011).

Previously, we investigated how the genotype and the environmental conditions during the growth season affect the composition of wild sea buckthorn berries from two subspecies, ssp. *rhamnoides* and ssp. *sinensis* (Kortesniemi et al., 2014). In the current study, we compared the metabolic profiles of sea buckthorn berries of two different cultivars, 'Terhi' and 'Tytti', collected from southern and northern Finland and Québec, Canada, using NMR metabolomics. The unique study design allows us to compare the effect of "terroir" or, in particular, of different geographical locations and related weather conditions on sea buckthorn berries with the same genetic background. The effect of growth location and related weather parameters on the berry composition were examined. Also, the metabolic differences between ripe and overripe berries were studied. The results add to the existing knowledge on factors affecting the composition and quality of sea buckthorn berries of different genotypes and origins.

2. Material and methods

2.1. Samples

Sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) berries of two Finnish cultivars, 'Terhi' (TTA-361) and 'Tytti' (TTA-362), were hand-picked from cultivation sites in Turku, Southern Finland (latitude 60° 23′ N, longitude 22° 09′ E, altitude 1 m), Kittilä, Northern Finland (68° 02′ N, 24° 37′ E, 210 m) and Québec, Canada (46° 47′ N, 71° 17′ W, 100 m) in four consecutive years from 2007 to 2010 (from Kittilä 2009 and 2010 only). The bushes were planted in Kittilä and Turku in 2003 and 2004, respectively. In Turku, the shrubs grow right on the shore of the Baltic Sea. In Québec, the one-year old bushes were received bare-root in May 2003, kept in pots, and planted in June 2004. Due to a very dry period in mid-July in 2010 in Québec, additional irrigation to the plants was temporarily applied. The berry samples were picked when optimally ripe, frozen and stored at −20 °C until analysis. The ripeness was evaluated by local experts. Overripe berries of season 2010 were also collected from Turku and Kittilä (11/26/2010 and 03/31/2011, respectively) and naturally frozen.

2.2. Meteorological data

The meteorological data were obtained from the Finnish Meteorological Institute (Helsinki, Finland) and from the National Climate Data and Information Archive of the Environment Canada (Fredericton, NB, Canada). Data from the weather stations of Turku Artukainen (latitude 60° 27′ N, longitude 22° 10′ E, altitude 8 m), Kittilä Pokka (68° 10′ N, 25° 47′ E, 275 m) and the Jean Lesage International Airport (46° 47′ N, 71° 23′ W, 74 m) were applied for Turku, Kittilä, and Québec, respectively. Values of various weather variables from the start of the growth season until harvest were calculated and applied in the multivariate analysis.

2.3. Chemicals

Acetone, formic acid and sodium hydroxide were purchased from J.T. Baker (Deventer, the Netherlands). Deuterium oxide (D_2O, 99.9 atom-% D) and 3-(trimethylsilyl)propionic-2,2′,3,3′-d_4 acid sodium salt (TSP, 98 atom-% D) were purchased from Sigma–Aldrich (St. Louis, MO).

2.4. Sample preparation and NMR analysis

The sample preparation and the NMR analyses followed the method described earlier (Kortesniemi et al., 2014). Briefly, the lyophilised acetone–water extracts of the seedless sea buckthorn berries were analysed in formic acid-buffered D_2O with 0.05% TSP (pH 2.70). Three technical replicates were performed to take the possible sample inhomogeneity into account. The NMR spectra were measured with a Bruker Avance 500 spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) equipped with a broadband inverse (BBI-5 mm-Zgrad-ATM) autotune probe. The proton spectra consisting of 320 scans were acquired with a 1D NOESY presaturation pulse program (*noesypr1d*).

2.5. Data processing and multivariate data analysis

The spectra were phase- and baseline-corrected and the chemical shifts were referenced to the TSP signal at 0 ppm with TopSpin 1.3 software (Bruker BioSpin GmbH, Rheinstetten, Germany)/Chenomx NMR Suite 8.1 Professional (Chenomx Inc., Edmonton, AB, Canada). A shim correction to a linewidth of 0.9 Hz was applied (Chenomx Processor). Possible variation between replicate samples and in chemical shifts was minimised as follows: the spectra were binned to 0.02 ppm-sized bins (Chenomx Profiler) and the data normalised to total spectral area. Technical replicates were averaged and variable-sized bins were created by combining equidistant bins in Excel. The custom bins contained only signals from single compounds whenever possible (Table 1). Bins containing no peaks or solvent peaks were excluded. Finally, a dataset of 22 objects and 110 variables was obtained, covering the spectral region of 0.55–9.19 ppm. The location- and weather-related parameters (Table 2) were combined as additional variables to the dataset (22×120). The overripe berries were omitted from the multivariate analyses.