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Variation of phenolic profile and antioxidant activity of North American highbush blueberry leaves with variation of time of harvest and cultivar

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ABSTRACT

The phytochemical composition of leaves varies with the stages of growth of a plant. Hence, during this study, moisture content, total phenolic content, total monomeric anthocyanins and antioxidant activity in terms of DPPH inhibition activity and FRAP in highbush blueberry leaves of two different cultivars Nelson and Elliot, harvested in May, July, September and October, were analysed and quantified using microwave assisted extraction with a solvent combination of ethanol and citric acid. Colour indices for respective powdered leaf samples were also analysed. It was observed that leaves of both the cultivars had a high amount of total phenolics in October (for Nelson 152.356 ± 3.369 and for Elliot 155.830 ± 2.103 mg GAE/g dry matter respectively). However, the blueberry leaves of the Nelson variety had the highest content of monomeric anthocyanins (1.202 ± 0.080 M 3-G equiv./g dry matter). The DPPH inhibition activity and FRAP for Elliot leaves were much higher than the Nelson leaves collected during May, July and September. However, Nelson leaves collected in October had higher DPPH inhibition activity and FRAP than the Elliot leaves. Total phenolic contents of the leaves were found to be strongly correlated to both DPPH inhibition activity and FRAP. Also strong positive correlation was observed between colour index "*a*" and total monomeric anthocyanin content, which implied an increase in redness of the leaves with an increase in the monomeric anthocyanin content which is experienced in the Fall season.

1. Introduction

Blueberry leaves are a rich source of phenolic compounds (Duy, 1999; Ehlenfeldt and Prior, 2001; Hokkanen et al., 2009; Piljac-Žegarac et al., 2009; Takeshita et al., 2009; Matsuo et al., 2010; Cyboran et al., 2013; Vyas et al., 2013). These compounds have been reported to have numerous valuable health beneficial effects including antioxidant properties, anti-diabetic effect and anti-bacterial effect (Grace et al., 2009; Park et al., 2011; Kelebek et al., 2013; Özşen and Erge, 2013). The extraction of phenolic compounds from parts of the plants, other than fruit, and addition of these to a variety of processed products can be an interesting option to ensure increased consumption of these health beneficial compounds. There are several reports on the beneficial effects of blueberry leaf extracts which support the idea of using blueberry leaves for the extraction of nutraceuticals (Skupień et al., 2006; Piljac-Žegarac et al., 2009; Vyas et al., 2013).

Biological and physico-chemical properties of biomaterials are governed by several environmental and biological factors. The concentration and bio-activity of their different phytochemicals vary as well. The composition, concentration, availability and bio-activity are some of the important factors deciding the application of a biological product. Some of the major factors affecting the phytochemicals' composition and concentration in blueberries (fruits) are cultivation practices including type of soil, water stress, climatic conditions, fertilizers or manure application practices, and also harvest time, stage of growth of the fruit during harvest and harvest practices (Routray and Orsat, 2011).

In fruits of different blueberry progenies, it was observed that total antioxidant capacity and total phenolic content were moderately heritable (Connor et al., 2002a,b; Scalzo et al., 2005) or in other cases from moderately to highly heritable (Scalzo et al., 2008). The genetic composition varies with different species, cultivars or varieties. Hence, the phytochemical compositions will be affected by variety and cultivar of the crop, which also differ with area of

Abbreviations: GAE, gallic acid equivalent; M 3-G, malvidin 3-glucoside; equiv, equivalent; ANOVA, analysis of variance; w, weight; v, volume; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing ability of plasma; RMSE, root mean square error; Prob, probability; AAE, ascorbic acid equivalent.

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cultivation and year of cultivation (Connor et al., 2002a,b). However, it has been observed that the genotype of a crop has a stronger effect than the environmental effects (Connor et al., 2002a,b). Similar observations have been made in case of different food materials and biological commodities such as walnut leaves, strawberry, blueberry, apple, peach, plum and kiwi fruit, where phenolic concentration and antioxidant activity varied with different cultivars (Häkkinen and Törrönen, 2000; Imeh and Khokhar, 2002; Kim et al., 2003; Pereira et al., 2007).

All the agronomic factors which affect the concentration of these phytochemicals in blueberries might affect the blueberry leaves as well. Leaves act as a basis to evaluate the health of the plants and also to detect nutrient deficiency in the plant during different stages of plant growth. In the last few decades there have been some post-harvest analytical reports regarding the antioxidant activity and phenolic content of leaves of lowbush blueberries, bilberries and other different varieties of berries (Duy, 1999; Witzell et al., 2003; Harris et al., 2007; Hokkanen et al., 2009; Martz et al., 2010; Matsuo et al., 2010; Hicks et al., 2012; Li et al., 2012), however post-harvest analytical reports on the leaves of North American highbush blueberries are relatively fewer (Ehlenfeldt and Prior, 2001; Kim et al., 2010; Janiuk et al., 2013; Routray and Orsat, 2014; Routray et al., 2014).

Ehlenfeldt and Prior (2001) have reported the antioxidant activity and phenolic content for leaves of various varieties of highbush blueberries, displaying the existence of an inherent variability of the phenolic content among different varieties of highbush blueberries. In another study, variation in DPPH inhibition activity along with phenolic content were analysed in leaves of highbush blueberry, varieties Bluecrop and Northland (Janiuk et al., 2013). However, there are no reports on the monomeric anthocyanin content or FRAP activity for blueberry leaf extracts. Also in the case of the North American highbush blueberries, there are no reports on the variation of phenolic compounds in the leaves, as a function of time of harvest through the growing season. Hence, during this study different analytical properties including total phenolic content, total monomeric anthocyanin content, antioxidant activity in terms of DPPH inhibition activity and FRAP, moisture content and colour indices were determined for leaves of two different varieties, Nelson and Elliot, collected concurrently during four different harvest times in July 2012, September 2012, October 2012 and May 2013 (which was the following spring). This selection of harvesting period was made not to represent the effects of one farming season as compared to another, but rather to only present the effect of different times of harvest of blueberry leaves. The main objectives of the study were (1) to compare the different analytical properties of the leaves of the two varieties, harvested at the same time; (2) to study the overall variation of the functional properties with different times of harvest and determine the possible interaction of variety of blueberry with time of harvest of the leaves; and (3) to analyse the effect of the studied factors and to decide on the best time of harvest of the blueberry leaves to obtain the highest level of desirable functional properties in case of these two blueberry varieties.

2. Materials and methods

2.1. Chemicals used

Analytical grade chemicals were used during the study. Ethanol was obtained from commercial alcohols (the industrial and beverage alcohol division of Greenfield Ethanol Inc., Brampton, Ontario, Canada), and DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, iron (III) chloride hexahydrate, gallic acid and TPTZ (2,4,6-tris (2-pyridyl)-s-triazine) were obtained from Sigma–Aldrich (St-Louis, MO, USA). Citric acid, sodium acetate, sodium carbonate, HCl, sodium acetate tri-hydrate, HPLC grade (high performance liquid chromatography) methanol, and potassium chloride were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Glacial acetic acid and ascorbic acid (Fisher Scientific, Nepean, Ontario, Canada) were also used during the analytical studies. Prerequisite concentrations of chemicals were prepared with the corresponding standards and HPLC grade water (prepared using Simplicity™ Water Purification System, Millipore, USA).

2.2. Collection of sample, processing, measurement of colour indices

Leaves of highbush blueberry (*Vaccinium corymbosum*), of varieties "Nelson" and "Elliot" were collected from a private farm, Bleuetière Sylvie Rémillard in Quebec during different periods which were July 2012, September 2012, October 2012 and May 2013. The leaves were immediately frozen at −18 °C and stored until drying. These leaves were dried using a freeze drier (Freezone® 2.5 l Freeze Dry System, Labconco Corporation, Kansas City, MO, USA) and powdered to decrease the particle size of the sample for further study. The initial weight of the fresh sample and the final weight of the dried samples were measured to calculate the moisture content of the leaves for all the samples.

The colour indices (*L*, *a*, *b*) of all dried powdered samples were measured using Colorimeter (Konica Minolta, Japan) and the samples were stored at −18 °C until being used for extraction and further analyses.

2.3. Microwave assisted extraction

The microwave assisted extraction method applied and the apparatus used during the study was similar to the extraction method previously used by our research group during two different studies reported on microwave extraction from blueberry leaves (Routray and Orsat, 2014; Routray et al., 2014). For each sample of powdered blueberry leaf, of specific variety, harvested during different periods, microwave extraction was replicated twice. The extract obtained after each extraction was used for total phenolics and total monomeric anthocyanin content quantification and determination of antioxidant activity as radical scavenging capacity of blueberry leaves' extract in terms of DPPH and FRAP.

2.4. Total phenolics quantification

Spectrophotometric method based on the procedure described by Singleton and Rossi (1965), and Waterhouse (2005) was used for total phenolics content quantification with Folin-Ciocalteu reagent and gallic acid standard. This method had already been used for total phenolic quantification of highbush blueberry leaves in terms of mg Gallic acid equivalent (GAE)/L extract solution during previous studies conducted by our research group (Routray and Orsat, 2014; Routray et al., 2014).

2.5. Total monomeric anthocyanins quantification

pH differential method was used for the determination of total monomeric anthocyanin content. The method adapted during current study was based on the procedure described by Giusti and Wrolstad (Giusti et al., 2005) and has been applied during previous studies on blueberry leaves (Routray and Orsat, 2014; Routray et al., 2014). Quantification of total monomeric anthocyanins was done in terms of malvidin 3-glucoside (M 3-G) which has been reported as one of the significantly present compound in blueberry leaves (Duy, 1999). Water was considered as the blank solution and total monomeric anthocyanin content was calculated using the