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Effect of growing location on anthocyanin content and total antioxidant capacity of haskap (*Lonicera caerulea* L.) berry: A preliminary investigation

*Aruma B.H.K. De Silva, H.P. Vasantha Rupasinghe**

Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, Dalhousie University, Truro, Nova Scotia, Canada

**Corresponding author: vrupasinghe@dal.ca*

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Abstract: The haskap has been successfully cultivated in many geographical zones in Canada. We investigated whether the latitude has any impact on the anthocyanin accumulation and total antioxidant capacity in the haskap berry. The measured quality attributes were compared with that of the blueberry. Haskap 'Aurora' berries were collected from Yukon, British Columbia, Saskatchewan, and Nova Scotia in Canada, and blueberries were collected from Nova Scotia. Cyanidin-3-*O*-glucoside (C3G), the most abundant anthocyanin of haskap berry, the total anthocyanins, the total polyphenols, the soluble solids (°Brix), the pH value, the total antioxidant capacity, and the moisture content were determined. The results revealed that the total anthocyanin, total phenolic content, and antioxidant capacity of the haskap range between 88 to 273 mg C3GE/100 g fresh weight (FW), 256 to 442 mg GAE/100 g of FW, and 27 to 52 μmol TE/g FW, respectively. The liquid chromatography-mass spectrometry (UPLC/MS) analysis revealed that the C3G contained in the haskap berry is highly dependent on the harvest stage. Among the samples, the haskap berry harvested on July 19, 2019, from the Yukon had the highest C3G content. Interestingly, the total anthocyanin content of the haskap berry is comparable to that of the lowbush blueberry, but higher than the highbush blueberry. A systematic multi-year experiment employing many cultivars and growing locations is recommended to better understand the latitude effect.

Keywords: blue honeysuckle; cyanidin-3-*O*-glucoside; latitude; blueberry; antioxidant

Haskap (*Lonicera caerulea* L.) is a recently commercialized berry crop in North America and is expanding rapidly partly due to the potential health-promoting properties of the berries (Rupasinghe et al. 2018; De Silva, Rupasinghe 2020). The haskap berry is unique due to the greater abundance of cyanidin-3-*O*-glucoside (C3G) when compared with other cultivated berry crops. Previous studies have shown that the total anthocyanin content, total phenolic content and antioxidant capacity are similar or higher in the haskap berry than that of most of the commonly consumed fruits, including the blueberry (Rupasinghe et al. 2012; Celli et al. 2014).

Environmental factors, including the temperature, light intensity, and photoperiod, showed an influence on the biosynthesis of anthocyanins in plants (Jaakola, Hohtola 2010). A higher concentration of polyphenols in berries grown in the northern latitudes depends on the genetic adaptation and climatic conditions (Jaakola, Hohtola 2010). The increased production of aromatic compounds of strawberries (*Fragaria ananassa* L.) was observed in the northern latitudes compared to the southern areas of Norway (Davik al. 2006). Another study indicated that the northern bilberry (*Vaccinium myrtillus*) contains a higher anthocyanin concentration compared to the southern

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counterparts (Lätti et al. 2008). However, the anthocyanidin concentration in bilberries has a strong influence on the genetics and climatic differences.

The effect of the growing location and latitude on haskap berry anthocyanins has not yet been reported upon. The overall goal of this preliminary investigation was to determine the effect of growing locations within Canada on the anthocyanin content and other quality attributes of the haskap 'Aurora' during the 2019 harvesting season.

MATERIAL AND METHODS

Sample collection. During the 2019 harvesting season, berries at three harvesting stages (early, mid and late) of the haskap 'Aurora' variety were collected from Yukon, British Columbia, Saskatchewan, and Nova Scotia of Canada. The haskap bushes were three to four years from planting. The locations of the haskap samples collected are given in Table 1 and Figure S1. The anthocyanin content of the haskap berries was compared with highbush and lowbush blueberries harvested at commercial maturity in Nova Scotia.

Extraction of the anthocyanin. The haskap berries (10 g) were blended with 90 mL of an extraction solvent (80% ethanol, 1% formic acid, 19% distilled water) using a Waring commercial laboratory blender for a minute. The mixture was then centrifuged at 3000×*g* for 10 min (Sorvail Legend Micro 21 R, Thermo Fisher Scientific Inc., Waltham, USA). The supernatant was collected into a measuring cylinder and preserved. An additional 40 mL of the extraction solvent was added to the pellets of the previous step and mixed using a vortex for a few seconds and placed in an ultrasonic bath of 20 kHz/1000 Watts (model 750D, VWR, West Chester, PA, USA), with a temperature of 25 °C for 20 min. After the extraction, the content was centrifuged at 3000×*g* for 10 min. The supernatants were combined, and the volume was adjusted to 150 mL using the same extraction solvent. This extract was used for the analysis of the total anthocyanin content, total phenolic content, total antioxidant capacity, and C3G content.

Determination of the total anthocyanin by the pH differential method. The total anthocyanin content of the berry berry samples was determined using the pH-differential method (AOAC method 2005.02). The diluted samples (1/200) in both a pH 1 and pH 4.5 buffer were prepared in duplicate, and the absorbance was measured at 520 nm and 700 nm using a microplate reader (Tecan Infinite® M200 PRO, Morrisville, NC, USA). The absorbance values (A) of the diluted samples were calculated using the following equation:

$$A = (A520 - A700) \text{ at pH } 1.0 - (A520 - A700) \text{ at pH } 4.5$$

The total anthocyanin content was calculated using the the equation:

$$\text{Total anthocyanin content} = \frac{A \times MW \times DF \times 1\,000}{\varepsilon \times L}$$

Table 1. The collection of haskap berry samples from various locations in Canada at different harvesting stages/dates

Province/ territory	Location	Latitude	Harvest stage	Harvest dates
Nova Scotia (NS)	New Canada	44°28'41.51'' N	H1 – early	July 10, 2019
			H2 – mid	July 15, 2019
			H3 – late	July 22, 2019
Nova Scotia (NS)	Stewiacke	45°08'32.41'' N	H1 – early	July 15, 2019
			H2 – mid	July 31, 2019
			H3 – late	August 02, 2019
Saskatchewan (SK)	Saskatoon	52°8'49.1028'' N	H1 – early	July 08, 2019
			H2 – mid	July 12, 2019
			H3 – late	July 16, 2019
British Columbia (BC)	Salmon Arm	50°42'7.9956'' N	H1 – early	June 10, 2019
			H2 – mid	July 12, 2019
			H3 – late	August 15, 2019
Yukon (YK)	Whitehorse	60°43'16.2768'' N	H1 – early	July 04, 2019
			H2 – mid	July 12, 2019
			H3 – late	July 19, 2019

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The molar extinction coefficient (ε); 26 900, the molecular weight of C3G (MW); 484.83 g/mol, the dilution factor (*DF*); the path length (*L*) (Lee et al. 2005).

Determination of the total phenolic content (TPC). The TPC in the haskap berry samples was determined using a Folin-Ciocalteu assay (Singleton et al. 1999) modified to be performed by using 96-well plates (Rupasinghe et al. 2008). Briefly, 20 µL of the diluted (1/20) haskap extract was mixed with 100 µL of the 0.2 N Folin-Ciocalteu reagent in the wells of the clear 96-well microplates (COSTAR 9017, Fisher Scientific, Canada) and left at room temperature for 5 min in the dark. Then, 80 µL of a 7.5% sodium carbonate solution was added and incubated for 2 h at room temperature before taking a reading at 760 nm using the microplate reader. A standard curve was prepared using 10 to 250 mg/L gallic acid, which was used to estimate the total phenolics in a mg gallic acid equivalence (GAE)/100 g of fresh weight (FW).

The total antioxidant capacity. The total antioxidant capacity of the haskap berry extracts was determined by a Ferric Reducing Antioxidant Power (FRAP) assay (Benzie, Strain 1996), as modified by Rupasinghe et al. (2008). The working reagent, consisting of 300 mM of an acetate buffer (pH 3.6), 20 mM of ferric chloride, 1 mM of a 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution (10 : 1 : 1, v/v/v), was freshly made and added (180 µL) to 20 µL of the haskap berry extract or the Trolox standard in a 96-well microplate. The absorbance values were measured at 593 nm using a microplate reader, after 6 min incubation at room temperature in the dark. The antioxidant capacity of the extracts was calculated based on the calibration curve of the 5–450 µM Trolox standards (µmol Trolox equivalent (TE)/100 g FW.

Moisture analysis. The moisture content of each sample was analyzed using a moisture analyser (model MF-50 A&D Store, Wood Dale, USA).

Total soluble solids measurements. Approximately 10 g of frozen haskap berries were crushed using a garlic press and the soluble solids value of the resulting juice was measured using a refractometer (model 300016, SPER SCIENTIFIC, Scottsdale, USA).

Ultra pressure liquid chromatography (UPLC) analysis of cyanidin-3-*O*-glucoside (C3G). C3G, the most abundant polyphenol compound of the haskap berry was quantified according to the method described by Rupasinghe et al. (2008) using ultra-pressure liquid chromatography (UPLC) coupled with electrospray ionization (ESI) and mass spectrometry (MS) (Waters, Milford, USA).

Statistical analysis. The experiment design was a completely randomized design with three replicates. The statistical analysis was performed using a two-way analysis of variance (ANOVA) using the Minitab statistical software (version 18.0) at a 5% significance level ($P < 0.05$). In order to compare the haskap and blueberry samples (Table 2), a statistical analysis was performed using a one-way analysis of variance (ANOVA) using the Minitab statistical software (version 18.0). For Table 3 and 4, the means were compared using Tukey's multiple means comparison at a $P < 0.05$ level. The two main factors were the location and harvesting stage. The means were compared by Tukey's multiple means comparison at a $P < 0.05$ level.

RESULTS AND DISCUSSION

Total soluble solids. The total solids measured as soluble solids ranged from 11% to 15.8% in the haskap berries and 8% to 9% in the blueberries, which agrees with previous studies (Thompson 2006; Rupasinghe et al. 2012). The lowbush blueberries contained significantly lower soluble solids compared to the haskap samples (Table 2). The highest soluble solids value (15.8%) was reported in the haskap samples collected from Saskatchewan and British Columbia at the H3 and H2 harvesting stages, respectively. The moisture content of the samples varied from 75% to 82% which was dependent on the harvesting date and growing location.

Total phenolic content (TPC). The total phenolic content was measured using a Folin-Ciocalteu assay. The current study indicated that the total phenolic content of the haskap berry ranged from 256 to 433 mg GAE/100 g of FW. The lowbush blueberry and highbush blueberry contained comparatively lower values, 347 mg GAE/100 g of FW and 158 mg GAE/100 g of FW, respectively (Table 2). Previous studies of the phenolic content of the haskap have shown 428 to 623 mg GAE/100 g of FW (Rupasinghe et al. 2012; Rupasinghe et al. 2018) and 575 to 903 mg GAE 100/g of FW (Rop et al. 2011). The total phenolic content of the highbush blueberry was significantly lower than that of the haskap and lowbush blueberry. Haskap berries have shown higher total phenolic values compared to other fruits, including the wild red raspberry, blackberry, and blueberry (Bakowska-Barczak et al. 2007; You et al. 2011).

Total anthocyanin content (TAC). The total anthocyanin content of the haskap berry samples,

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Table 2. Total anthocyanin content (TAC), total phenolic content (TPC), total antioxidant capacity (FRAP values), cyanidin-3-*O*-glucoside (C3G) content, moisture, total soluble solids (°Brix) and pH values of the haskap berry and blueberry

Location/ sample	Harvest stage	TAC (mg C3GE/ 100 g FW)	TPC (mg GAE/ 100 g FW)	FRAP (µmol TE/ g FW)	C3G (mg/ 100 g FW)	Moisture (%)	Soluble solids (%)	pH
Haskap berry								
New Canada, NS	H1	139^{bc}	295^{ab}	31.2^{b}	47.5^{bcd}	82.5^{abc}	11.9^{ef}	2.9^{cd}
	H2	130^{bc}	256^{ab}	26.7^{b}	47.5^{bcd}	84.3^{a}	12.5^{bcdef}	2.9^{bcd}
	H3	189^{ab}	353^{a}	40.0^{ab}	84.5^{bcd}	82.1^{abcd}	12.5^{bcdef}	3.1^{abcd}
Stewiake, NS	H1	184^{ab}	373^{a}	38.6^{ab}	79.0^{bcd}	82.2^{abcd}	12.1^{cdef}	3.1^{abcd}
	H2	204^{ab}	409^{a}	41.5^{ab}	106^{ab}	81.5^{abcd}	12.9^{abcdef}	3.1^{abc}
	H3	175^{ab}	366^{a}	34.9^{ab}	90.5^{b}	81.1^{abcde}	13.7^{abcde}	3.1^{abc}
Saskatoon, SK	H1	143^{bc}	385^{a}	37.5^{ab}	63.5^{bcd}	78.3^{def}	14.2^{abcde}	3.1^{abc}
	H2	165^{bcd}	405^{a}	36.3^{ab}	87.5^{bcd}	77.3^{ef}	15.0^{abcd}	3.2^{ab}
	H3	193^{ab}	388^{a}	43.3^{ab}	122^{ab}	78.5^{cdef}	15.8^{a}	3.2^{a}
Salmon Arm, BC	H1	94.6^{bc}	396^{a}	30.3^{b}	36.0^{bcd}	80.4^{abcdef}	14.8^{abcde}	3.1^{abcd}
	H2	88.3^{bc}	341^{a}	27.4^{b}	29.3^{bcd}	78.5^{bcdef}	15.8^{ab}	3.2^{abc}
	H3	149^{abc}	364^{a}	41.6^{ab}	63.0^{bcd}	76.5^{f}	15.5^{abc}	3.2^{abc}
Whitehorse, YK	H1	183^{ab}	384^{a}	40.4^{ab}	105^{ab}	79.7^{bcdef}	12.8^{abcdef}	2.9^{cd}
	H2	166^{abc}	379^{a}	40.4^{ab}	90.0^{bcd}	79.8^{abcdef}	11.6^{defg}	2.8^{d}
	H3	273^{a}	433^{a}	52.0^{a}	197^{a}	75.5^{f}	13.9^{abcde}	2.9^{bcd}
Blueberry								
Lowbush NS	n/a	150^{bc}	347^{a}	26.8^{b}	3.38^{cd}	81.3^{abcde}	9.9^{fg}	3.3^{a}
Highbush NS	n/a	57.4^{c}	158^{b}	26.3^{b}	1.65^{d}	82.7^{ab}	8.9^{g}	3.0^{abcd}

n/a – not applicable, since the lowbush and highbush blueberry were collected at commercial harvest; the data were analysed using a one-way ANOVA and Tukey's mean comparison using the Minitab statistical software; different superscript letter in each column represents statistically significant ($P \leq 0.05$) differences; TAC, TPC, FRAP and C3G values are calculated based on the fresh weight (FW) of the samples

determined by the pH differential method, ranged between 88 to 273 mg C3GE/100 g of FW, which is similar to previous studies (Rupasinghe et al. 2012). These values are higher than other fruit sources, including the raspberry (Chen et al. 2013) and blueberry (Skrede et al. 2000). The anthocyanin content was significantly higher in the haskap berries compared to the highbush blueberry (Table 2). The UPLC analysis revealed that the haskap berry samples contained a C3G between 29 to 197 mg/100 g of FW. These values are in agreement with previous studies, which revealed a C3G concentration of 221 mg/100 g of FW and 170 mg/100 g of FW in Polish-bred Zielona and Canada-bred Borealis cultivars, respectively.

Total antioxidant capacity. The antioxidant capacity, measured by FRAP assay, ranged between 27 to 52 µmol TE/g of FW (Table 2), which agrees with previous reports, which showed values ranging from 8 to 113 µmol of TE/g of FW (Sánchez-Moreno 2002; Rop et al. 2011). In a previous report, the blueberry (*Vaccinium angustifolium* L.) showed a lower antioxidant capacity, 27 µmol TE/g of FW compared with haskap berries (Rupasinghe et al. 2012). In general, the FRAP value was not significantly influenced by the harvesting dates or the growing location, with a few exceptions. The pH value varied between 2.8 to 3.2 in the haskap and 3 to 3.3 in the blueberry.

Among the tested samples, the haskap 'Aurora' berries harvested on July 19, 2019, from the Yukon had the highest C3G content. However, a significant difference was not observed in the phenolic content based on the growing locations. This suggested that the latitude may have an influence on the an-

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Table 3. The *P*-values of the main factors (Location and Harvesting stage) and interaction effect (Location × Harvesting stage) of the parameters of the haskap berry analysed using the two-way ANOVA

Source of variation	TAC	TPC	FRAP	C3G	Moisture	Soluble solids	pH
Location	**0.001**[b]	**0.01**[b]	**0.008**[b]	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Harvesting stage	**0.006**[b]	0.597	**0.006**[b]	**0.001**[b]	**0.002**[b]	**0.036**[b]	**0.048**[b]
Location × Harvesting stage	0.283	0.542	0.184	0.22	0.025	0.693	0.798

TAC – total anthocyanin content; TPC – total phenolic content; FRAP – Ferric reducing antioxidant power; C3G – Cyanidin-3-*O*-glucoside; [b]significant effects that need a multiple means comparison are shown in bold ($P < 0.05$)

thocyanin accumulation; however, the harvesting date or harvesting maturity is also an influencing factor of the anthocyanin content. Previous reports found that the proportions of certain anthocyanins have shown diversity between latitudes and between plants with different parental origins (Åkerström et al. 2010). A study on the bilberry (*V. myrtillus* L.) demonstrated that the sugar and antioxidant levels were higher in northern clones compared to southern counterparts due to both the genes and environmental conditions (Lätti et al. 2008; Åkerström et al. 2010; Uleberg et al. 2012). The two-way ANOVA results indicated that the total phenolic content (TPC) is significant as the main effect per the location, but not by the harvesting stage (Table 3). Both main factors; the location and harvesting stage significantly influenced the TAC, total antioxidant capacity (FRAP), and almost all the other quality attributes measured in this study (Table 3). Statistically, the late harvesting stage (H3) showed a significantly higher Brix, pH, TAC, C3G, and total antioxidant capacity (FRAP) (Table 4). Moreover, the highest moisture content was shown in the H1 and H2 harvesting stages (Table 4). Furthermore, in terms of the location, the highest TPC, TAC, and total antioxidant capacity were found in the haskap berries collected from locations such as the Yukon (YK), Saskatchewan (SK) and the second location in Nova Scotia (NS2) (Figure 1).

CONCLUSION

Overall, the growing location and associated environmental factors have a significant impact on the anthocyanin content, phenolic content and antioxidant capacity of the haskap berry. The results demonstrated that the total anthocyanin content, total phenolic content, and antioxidant capacity of haskap berries varied from 88 to 273 mg C3GE/100 g of fresh weight (FW), 256 to 442 mg GAE/100g of FW, and 27 to 52 μmol TE/g of FW, respectively. Based on the UPLC/MS analysis, the C3G content in the haskap berries is highly dependent on the harvesting date. The haskap berries harvested from Yukon on July 19, 2019, showed the highest C3G content. Furthermore, the total anthocyanin content of the haskap is comparable to that of the lowbush blueberry, but higher than the highbush blueberry. However, to better understand the advantages of growing haskap in higher latitudes to obtain superior berry qualities, such as higher accumulated anthocyanins, a systematic multi-year experiment employing many harvesting maturity stages, a number of selected cultivars and a wide range of latitudes of growing locations are recommended. Additional measurements regarding the growing locations, such as temperature, water stress, and light duration (photoperiod), light quality (intensity), could also be investigated to understand their influence on the fruit's qualities.

Table 4. The effect of the harvesting stage on the berry quality parameters of the haskap

Harvesting stage	Soluble solids (%)	pH	TAC (mg C3GE/ 100 g FW)	UPLC-C3G (mg/100 g FW)	FRAP (μmol TE/g FW)	Moisture (%)
H1 – early	13.14[b]	3.01[b]	148.4[b]	66.2[b]	35.6[b]	80.6[a]
H2 – mid	13.54[ab]	3.06[ab]	150.5[b]	72.1[b]	34.5[b]	80.3[a]
H3 – late	14.28[a]	3.11[a]	195.7[a]	111.5[a]	42.4[a]	78.7[b]

TAC – total anthocyanin content; TPC – total phenolic content; FRAP – Ferric reducing antioxidant power; C3G – Cyanidin-3-*O*-glucoside; the means were compared using Tukey's multiple means comparison

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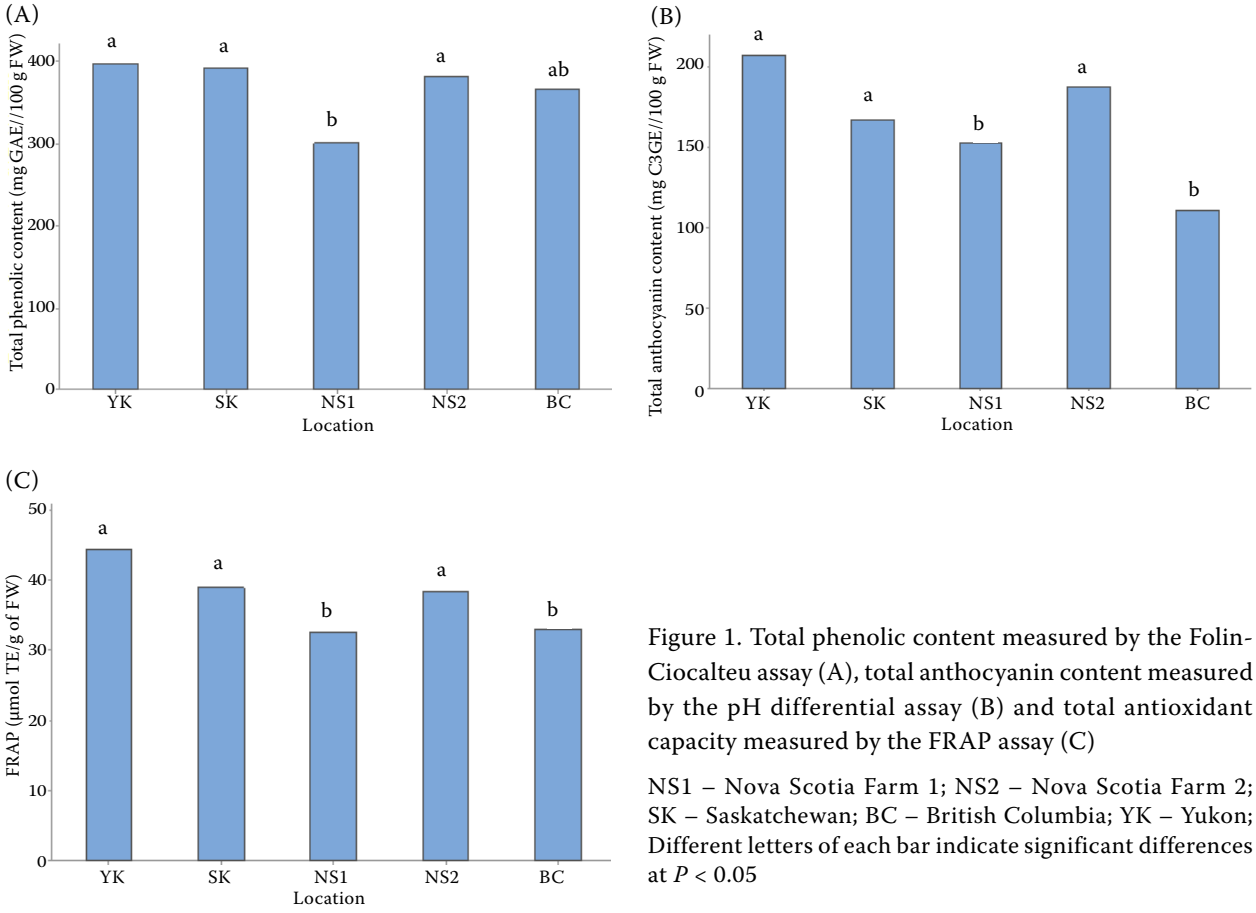

Figure 1. Total phenolic content measured by the Folin-Ciocalteu assay (A), total anthocyanin content measured by the pH differential assay (B) and total antioxidant capacity measured by the FRAP assay (C)

NS1 – Nova Scotia Farm 1; NS2 – Nova Scotia Farm 2; SK – Saskatchewan; BC – British Columbia; YK – Yukon; Different letters of each bar indicate significant differences at $P < 0.05$

In addition to anthocyanins, other polyphenols and iridoids need to be investigated.

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