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Lingonberry (*Vaccinium vitis-idaea* L.) grown in the Pacific Northwest of North America: Anthocyanin and free amino acid composition

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ABSTRACT

Lingonberries and their products are popular and generally accessible in Europe, though in the US they are uncommon and considered a minor berry/fruit crop. The on-going interest in potential health benefits from berry consumption has heightened interest in broadening the selection of berry/fruit crops in the US. This study measured total phenolics, total tannins, complete anthocyanin content, and total (and individual) free amino acid composition for each of five lingonberry cultivars. Cultivars Ida, Koralle, Linnea, Sanna, and Sussi were grown in Oregon, USA, and had only been evaluated previously for their horticultural traits. All five cultivars contained the three anticipated anthocyanins (by HPLC): cyanidin-3-galactoside (main anthocyanin found in these berries), cyanidin-3-glucoside, and cyanidin-3-arabinoside. These lingonberries' total anthocyanin content ranged from 27.4 ('Linnea') to 52.6 ('Ida') mg/100 g fw. They contained 22 free amino acids (FFAs) and total FAAs ranged from 28.92 ('Sanna') to 70.38 ('Koralle') mg/100 g fw. Asparagine (ASN) was the leading FAA (22–34% of the total FAAs) for all five cultivars. This is the first report on lingonberry FAA content.

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1. Introduction

Lingonberries (also known as cowberries; family Ericaceae) are not as popular or readily available in the United States (US) marketplace as blueberries (*Vaccinium corymbosum* L., *V. angustifolium* L., etc.) or cranberries (*V. macrocarpon* L.), which also belong to the genus *Vaccinium*. Unlike Europe, lingonberries are categorized in the US with other new berry/fruit crops (also referred to as minor berries/crops) like black and red currants, chokeberries, cloudberries, crowberries, elderberries, gooseberries, etc. (Hjalmarsson & Ortiz, 2001) and are not collected, purchased, or consumed to any significant extent (Hakkinen, Karenlampi, Heinonen, Mykkanen, & Torronen, 1999a; Hjalmarsson & Ortiz, 2001; Heinonen 2007). Canadian consumption of lingonberries is also higher than that of the US (Bakowska-Barczak, Marianchuk, & Kolodziejczyk, 2007; Debnath & Sion, 2009; Kalt et al., 2008).Yet there is interest, and a new program to educate and introduce 'novel' berries to US consumers through a USDA initiative (Healthier US School Challenge, HUSSC) aims to bring a wider array of fresh fruits and vegetables onto the menus of schools.

North American wild lingonberries are found throughout much of Canada, in Alaska and eight other northern US states (Connecticut, Maine, Massachusetts, Michigan, Minnesota, New Hampshire, Wisconsin, and Vermont) (Hjalmarsson & Ortiz, 2001; USDA Plants Database, 2010). Despite little

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commercial attention in the US, there is an estimated 20 acres of lingonberry production in Oregon and Washington (Burt & Penhallegon, 2003). Their novelty and the promising health benefits a diversity of phenolic-rich minor berries could provide have increased recent efforts to successfully develop and expand their commercial production (Finn & Mackey, 2006; Lee & Finn, 2007).

The potential health benefits attributed to lingonberries (well reviewed by Heinonen, 2007; references therein), are due to their structurally diverse phenolics (Andersen, 1985; Bakowska-Barczak et al., 2007; Ek, Kartimo, Mattila, & Tolonen, 2006; Hakkinen et al., 1999a,b; Hellstrom and Mattila, 2008; Hellstrom, Torronen, and Mattila, 2009; Kahkonen, Heinamaki, Ollilainen, & Heinonen, 2003; Kalt et al., 2008; Kylli et al., 2011; Maatta-Riihinen, Kahkonen, Torronen, & Heinonen, 2005), vitamins (Hakkinen et al., 1999a), and omega-3 fatty acid (Bere, 2007). Lingonberry phenolics (specifically anthocyanins and flavonol-glycosides metabolism) have been monitored in human subjects with demonstrated bioavailability (Erlund et al., 2003; Lehtonen, Rantala, Suomela, Viitanen, & Kallio, 2009; Lehtonen, Lehtinen, Suomela, Viitanen, & Kallio, 2010).

The majority of lingonberry phenolic research has been conducted in Finland, Sweden, and Norway (as listed in the reference section of this paper). Though interest in lingonberry and other minor berries has brought research attention to its commercial potential, it remains a challenge for lingonberries to gain acceptance by US consumers. To the best of our knowledge, there are no reports on lingonberry free amino acid quantification; only one paper (Burroughs, 1960) exists reporting a few free amino acids found in lingonberry. The objective of this study was to evaluate fruit maturity indices, and detail the anthocyanin profiles and free amino acid components of US grown lingonberries.

2. Materials and methods

2.1. Plant material and fruit maturity index

Frozen fruit from lingonberry cultivars (Ida, Koralle, Linnea, Sanna, and Sussi; $n = 5$) were obtained from a research plot in Corvallis, OR, USA (Finn & Mackey, 2006). These cultivars along with a number of selections from breeding program were being evaluated for their horticultural characteristics and commercial potential. Most lingonberries will produce a summer and fall crop in regions such as Oregon that have a long growing season. The fruit for this evaluation was from the 2nd, late summer/fall crop. The details of this research plot design are described in Finn and Mackey (2006). Fruit was collected at commercial ripeness in 2005 and stored at −20 °C until extraction and analysis. One-hundred berry weights, pH, TA (titratable acidity; expressed as g citric acid/100 g), and % soluble solids (°Brix) were conducted as described in Lee and Finn (2007).

2.2. Reagents, chemicals, and standards

All chemicals, reagents, and standards used in this study were analytical or HPLC grade from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA), unless indicated otherwise.

2.3. Extraction and sample preparation

All berries were liquid nitrogen (N; Norco Inc., Nampa, ID, USA) powdered and extracted as described in Lee and Wrolstad (2004) using an IKA M20 Universal Mill (IKA Works Inc., Wilmington, NC, USA). Briefly, 10 g of liquid N berry powder were extracted with 100% acetone, subsequently two additional extractions were done with 70% aqueous acetone (30:70 = water:acetone, v/v). Acetone was evaporated (using a RapidVap Vacuum Evaporation System set at 40 °C under vacuum; Labconco Corp., Kansas City, MO, USA) and extracts were redissolved in water (25 mL). These chemically extracted samples were used for all phenolic analysis. A second set of liquid N powdered berries was water extracted as described in Lee and Schreiner (2010) for free amino acid (FAA) analysis. Both extracts were stored at −80 °C until analysis.

2.4. Spectrophotometric methods used for phenolic analyses

Total anthocyanins (TACY), total phenolics (TP), and total tannins (TT) were analyzed for all extracts as described in Lee, Durst, and Wrolstad (2005), Waterhouse (2002), and Sarneckis et al. (2006). Absorbances were measured at 520 and 700 nm for TACY, 765 nm for TP, and 280 nm for TT. TACY was expressed as mg cyanidin-3-glucoside/100 g, TP was expressed as mg gallic acid/100 g, and TT was expressed as mg epicatechin/100 g. A SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA) was used for all three measurements. All measurements were conducted in duplicates. All samples were expressed in mg/100 g of whole berries (fresh weight, fw), but will be referred to as mg/100 g for conciseness.

2.5. HPLC conditions for anthocyanin and FAA analyses

HPLC/DAD (for identification and quantification) and HPLC/DAD/MS (for identification) analysis for anthocyanin did not differ from our earlier published methods (Lee & Finn, 2007; Lee, Rennaker, & Wrolstad, 2008). Briefly, an Agilent HPLC 1100 (Agilent Technologies Inc., Palo Alto, CA, USA) was used for this investigation. MS was used when needed. All quantification was done on the HPLC/DAD monitored at 520 nm. Anthocyanins were expressed as cyanidin-3-glucoside (Polyphenols Laboratories AS, Sandnes, Norway). Anthocyanin peaks were identified based on retention time, UV-VIS spectra, external standards (when available), mother and daughter ions' information, and prior published research (Kahkonen et al., 2003; Latti, Riihinen, & Jaakola, 2011).

FAAs were determined as described previously (Lee, Keller, Rennaker, & Martin, 2009; Lee & Schreiner, 2010), using HPLC/DAD monitored at 338 and 262 nm with in-line derivatization by OPA (o-phthalaldehyde) and FMOC (9-fluorenylmethyl chloroformate). Individual FAAs were identified against purchased external standards and expressed as mg of each amino acid/100 g. Internal standards of norvaline and sarcosine were used. Individual FAAs were summed for a total FAA amount, and essential FAAs were also calculated.

2.6. Statistical analysis

Statistical analysis was conducted using Statistica for Windows version 7.2 (StatSoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) was conducted and Tukey's Honest Significant Difference (HSD) determined the significant difference among the cultivars ($\alpha = 0.05$).

3. Results and discussion

3.1. Fruit maturity index

Fruit maturity index is summarized in Table 1. The five cultivars' pH range was 2.74–2.90, TA range was 1.84–2.73 g of citric acid/100 g, and % soluble solids of 15.1–19.9 °Brix. These values are similar to what was reported in Finn and Mackey (2006), who assessed these cultivars from the 2003 season. These pH values were also similar to that (2.67) reported by Viljakainen, Visti, and Laakso (2002). We found 'Ida' had the largest berries (100 berry weight was 43 g) and the lowest % soluble solids of 15.1 °Brix. Finn and Mackey (2006) also observed that 'Ida' had larger berries and lower % soluble solids content (15.1°Brix) compared to the other 21 evaluated cultivars. And as 'Ida' has shown to have one of the higher fruit yields (Finn & Mackey, 2006; Penhallegon, 2006), it is a likely candidate for commercial production.

3.2. Anthocyanin

Three anthocyanins were found in each of the five samples (Table 2), and in order of elution (% proportion of individual anthocyanins) were: cyanidin-3-galactoside (79%), cyanidin-3-glucoside (10%), and cyanidin-3-arabinoside (11%). Individual anthocyanin content and proportion to that of the total sum are listed by cultivar in Table 2. Lingonberries contained only cyanidin based anthocyanins, as was found in other reports (Ek et al., 2006; Latti et al., 2011; Lehtonen et al., 2009; Kahkonen et al., 2003). Cyanidin-3-glactoside (>68% of total anthocyanins) was the main anthocyanin, similarly noted by several others (Andersen, 1985; Bakowska-Barczak et al., 2007; Ek et al., 2006; Kahkonen et al., 2003; Latti et al., 2011). These US grown lingonberries contained similar proportions of cyanidin-3-glucoside and cyanidin-3-arabinoside. Four cultivars had slightly greater concentrations of cyanidin-3-arabinoside than cyanidin-3-glucoside, but 'Koralle' had the opposite trend with more cyanidin-3-glucoside than cyanidin-3-arabinoside. Overall, cyanidin-3-glucoside and cyanidin-3-arabinoside made up 15–33% of the total anthocyanins. Others reported (Andersen, 1985; Kahkonen et al., 2003; Latti et al., 2011) less than 2% of their lingonberry anthocyanin was cyanidin-3-glucoside and cyanidin-3-arabinoside. Our berry samples had an identical profile to what was observed by Kahkonen et al. (2003) without the additional minor peaks others have seen (Ek et al., 2006; Latti et al., 2011).

Lehtonen et al. (2009) reported only galactoside and glucoside of cyanidin in their wild Finnish lingonberry samples. Delphinidin-3-glucoside was not present in our samples, although minor levels of this anthocyanin have been noted in wild Norwegian lingonberry by Andersen (1985). Latti et al. (2011) found two additional unknown cyanidin based anthocyanins. Small amounts of peonidin derived anthocyanins have also reported (Koponen, Happonen, Mattila, & Torronen, 2007; Maatta-Riihinen, Kamal-Eldin, Mattila, Gonzales-Paramas, & Torronen, 2004).

Our anthocyanin levels by HPLC ranged from 27.4 ('Linnea') to 51.6 ('Ida') mg/100 g (Table 2), slightly below lingonberries from a Finnish marketplace – 68.0 mg/100 g fw and 77.5 mg/100 g fw (Kahkonen et al., 2003; Koponen et al., 2007), but well below wild Norwegian lingonberries (174 mg/100 g fw) reported by Andersen (1985) and wild Finnish lingonberries (130.3 mg/100 g fw) reported by Maatta-Riihinen et al. (2004) (all determined by HPLC).

TACY followed the same trend as prior measurements of anthocyanins determined by HPLC (Lee & Finn, 2007; Lee et al., 2008). TACY values ranged from 17 to 33 mg/100 g by pH differential. 'Ida' continued to produce the highest level of TACY (33 mg/100 g fw) in 2005 growing season compared to the other cultivars as reported by Finn and Mackey (2006), who found 44 mg/100 g fw (also by pH differential method) in 2003 grown samples. Debnath and Sion (2009) reported low anthocyanins in 'Ida' and 'Sanna' berries, which were, respectively, 4 and 5 mg/100 g fw [recalculated as cyanidin-3-glucoside, since they determined their values as petunidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside], determined by pH differential method. The low amounts they found (5–26 mg/100 g fw from 18 lingonberry genotypes) might have been due to greenhouse growing conditions (Debnath & Sion, 2009) and the different berry extraction method they employed.

Overall, the lingonberries in our study (38.2 mg/100 g by HPLC and 24 mg/100 g by pH differential) had concentrations of anthocyanins lower than other minor berry crops, like elderberries (207.7–842.6 mg cyanidin-3-glucoside/100 g fw determined by identical HPLC condition and 106–444 mg cyanidin-3-glucoside/100 g fw by pH differential; Lee & Finn, 2007). They were also lower than other *Vaccinium* crops such as cranberries (43–118 mg cyanidin-3-rutinoside/100 g fw by

Table 1 – Fruit maturity values of the five lingonberry cultivars examined.

Cultivars	Ida	Koralle	Linnea	Sanna	Sussi	Mean ± SE
100 Berry weight (g)	43 b	22 a	22 a	28 a	28 a	29 ± 3
pH	2.74 b	2.90 e	2.70 a	2.83 d	2.80 c	2.79 ± 0.02
TA (g of citric acid/100 g)	1.96 b	1.84 a	2.73 e	2.16 c	2.30 d	2.20 ± 0.10
% Soluble solids (°Brix)	15.1 a	18.5 c	19.9 d	17.8 b	17.7 b	17.8 ± 0.5

Means followed by the same letter within each row are not significantly different (Tukey's HSD). Values after ± are standard errors (SE). TA = titratable acidity.

Table 2 – Anthocyanin profiles and totals by HPLC (expressed as cyanidin-3-glucoside), TACY by pH differential (expressed as cyanidin-3-glucoside), TP by Folin–Ciocalteu (expressed as gallic acid), and TT by methylcellulose precipitation (expressed as epicatechin) methods. Values in parenthesis are proportion of the individual anthocyanins in %. Units are in mg/100 g.

Cultivars	Ida	Koralle	Linnea	Sanna	Sussi	Mean ± SE
By HPLC						
Cyanidin-3-galactoside	39.3 (76)	18.7 (68)	22.8 (83)	35.5 (82)	34.8 (85)	30.2 ± 2.7
Cyanidin-3-glucoside	5.1 (10)	5.2 (19)	1.9 (7)	3.8 (9)	3.0 (7)	3.8 ± 0.4
Cyanidin-3-arabinoside	7.2 (14)	3.8 (14)	2.8 (10)	3.9 (9)	3.2 (8)	4.2 ± 0.5
Total	51.6 d	27.7 a	27.4 a	43.2 c	42.0 b	38.2 ± 3.1
By spectrophotometric methods						
TACY	33 e	19 b	17 a	27 d	26 c	24 ± 2
TP	503 b	431 a	660 e	638 d	601 c	566 ± 29
TT	435 b	342 a	645 c	598 c	564 c	517 ± 38

Means followed by the same letter within each row are not significantly different (Tukey's HSD). Values after ± are standard errors (SE). TACY = total anthocyanins. TP = total phenolics. TT = total tannins.

spectrophotometric method; Viskelis et al., 2009), or either wild or cultivated blueberries (101–400 mg cyanidin-3-glucoside/100 g fw by pH differential; Lee, Finn, & Wrolstad, 2004), but slightly higher than gooseberries (20 mg cyanidin-3-glucoside/100 g fw by pH differential; Hummer & Lee, 2006).

'Linnea' fruit contained the most TP (660 mg/100 g) and TT (645 mg/100 g) of the cultivars compared here. 'Koralle' fruit contained significantly lower anthocyanins (27.7 mg/100 g), TP (431 mg/100 g), and TT (342 mg/100 g). Together, these lingonberries had TP ranging from 431 to 660 mg/100 g, compared to blueberries (367–1286 mg/100 g fw; Lee et al., 2004), cranberries (199–260 mg/100 g fw; Viskelis et al., 2009), or elderberries (277–582 mg/100 g fw; Lee & Finn, 2007) all by the same Folin–Ciocalteu method. Although, 'Sussi' is typically only used as a pollinizer cultivar (Penhallegon, 2006), it still had comparable levels of anthocyanins (42 mg/100 g), TP (601 mg/100 g), and TT (564 mg/100 g) to the other four cultivars (Table 2).

3.3. *FAA composition*

Twenty-two FAAs (abbreviations listed with corresponding full FAA names in Table 3; ASP, GLU, ASN, SER, GLN, HIS, GLY, THR, CIT, ARG, ALA, GABA, TYR, VAL, MET, TRP, PHE, ILE, LEU, LYS, HYP, and PRO in the order of elution) were found in each lingonberry cultivar (Table 3). FAAs ranged from 28.92 mg/100 g ('Sanna') to 70.38 mg/100 g ('Koralle') and essential FAAs ranged from 2.39 mg/100 g ('Sussi') to 5.83 mg/100 g ('Koralle'). 'Koralle' had the highest levels of total FAAs (70.38 mg/100 g) and essential FAAs (5.83 mg/100 g). The main FAA in all the cultivars was ASN. 'Ida' and 'Koralle' berries had ASN, GLN, ARG, GABA, and HYP, as the top five FAAs. 'Linnea' had ASN, SER, GLN, ARG, and GABA as the top FAAs. 'Sanna' and 'Sussi' had ASN, GLN, ARG, GABA, and PRO. Only 'Koralle' had quantifiable levels of MET.

Surprisingly very little work has been done on FAA content of fruit, beyond wine grapes (Lee et al., 2009; Lee & Schreiner, 2010). Burroughs (1960) found the common FAAs of Scottish grown lingonberries were ASP, GLU, ASN, SER, GABA, with another 12 unnamed amino acids, though unfortunately he reported no concentrations. Burroughs (1960) identified these amino acids by paper chromatography and visual relative amount rating. Those Scottish lingonberries also contained 1-aminocyclopropane-1-carboxylic acid (Burroughs, 1960), but our examination for common FAAs did not find this amino acid in these lingonberry samples.

Perez, Rios, Sanz, and Olias (1992) reported 'Chandler' strawberries contained 54.4 mg/100 g fw of FAA with Van Gorsel, Li, Kerbel, Smits, and Kader (1992) finding a similar 51.1 mg/100 mL fw of FAA in 'Douglas' strawberries. In apples, a range of 4.6–102.8 mg/100 g fw ('Glockenapfel', 'Collaos', 'Raxao', 'Meana', and 'Red Delicious') of FAA has been reported (Ackermann, Fischer, & Amado, 1992; Gomis, Lobo, Alvarez, & Alonso, 1990; Van Gorsel et al., 1992). In the FAA work that has been published, lingonberries are comparable to apples, but are not a conspicuous source of FAAs when judged against other fruit or their resulting products, including tomatoes (357.9 mg/100 mL; Kader, Stevens, Albright, & Morris, 1978), orange juices (180.8–300.2 mg/100 mL; Gomez-Ariza, Villegas-Portero, & Bernal-Daza, 2005), 'Roysum' plum (156.5 mg/100 mL; Van Gorsel et al., 1992), 'Desert Gold' peach (135.8 mg/100 mL; Van Gorsel et al., 1992), 'Red Delight' nectarine (146.9 mg/100 mL; Van Gorsel et al., 1992), or unnamed black seedless table grapes (227.7 mg/100 mL; Van Gorsel et al., 1992). More reports on FAA by Van Gorsel et al. (1992) were 'Hayward' kiwi (54.4 mg/100 mL), 'd'Anjou' pear (27.8 mg/100 mL), and 'Red Delicious' apple (23.6 mg/100 mL).

4. Conclusion

The Pacific Northwest of North America is suitable to grow lingonberries with fruit quality equivalent to more established growing regions like Finland. 'Ida' might be the best to grow in this region based on horticultural and fruit quality traits (i.e. vigor, yield, berry size, color, etc.) described by Finn and Mackey (2006). In this study, we found it had a large berry size and the highest anthocyanin content, relatively high TP and TT. But, 'Ida' had the lowest amount of total FAAs. 'Koralle' was also recommended for possible cultivar for commercial planting by Finn and Mackey (2006), and although we found 'Koralle' had relatively small berries, low TA, anthocyanins, TP, and TT, it also had high % soluble solids, FAAs,

Table 3 – Free amino acid (FAA) composition of lingonberries. Water extracted samples were analyzed by HPLC/DAD. Units are in mg of each free amino acid/100 g fw. All identifications were described previously (Lee et al., 2009; Lee and Schreiner, 2010).

Cultivars	Ida	Koralle	Linnea	Sanna	Sussi	Mean ± SE
Aspartic acid, ASP	0.57	1.11	1.62	0.47	0.70	0.90 ± 0.14
Glutamic acid, GLU	0.99	1.15	0.97	0.91	0.81	0.97 ± 0.04
Asparagine, ASN	10.18	23.89	13.78	6.50	8.25	12.52 ± 2.06
Serine, SER	1.72	2.82	2.66	0.91	1.01	1.82 ± 0.27
Glutamine, GLN	3.15	5.35	5.79	2.16	1.92	3.67 ± 0.54
Histidine, HIS	0.40	1.13	0.74	0.57	0.46	0.66 ± 0.09
Glycine, GLY	0.14	0.27	0.22	0.11	0.15	0.18 ± 0.02
Threonine, THR	0.53	0.97	1.35	0.43	0.51	0.76 ± 0.12
Citrulline, CIT	t	0.98	t	0.21	0.22	0.28 ± 0.12
Arginine, ARG	2.54	6.51	8.83	6.10	7.32	6.26 ± 0.70
Alanine, ALA	0.65	1.29	1.54	0.63	0.73	0.97 ± 0.13
γ-Aminobutyric acid, GABA	2.91	6.90	6.72	3.30	3.59	4.69 ± 0.59
Tyrosine, TYR	1.29	2.11	1.46	0.69	0.71	1.25 ± 0.18
Valine, VAL	1.30	1.53	0.90	0.66	0.59	1.00 ± 0.12
Methionine, MET	t	0.44	t	t	t	0.09 ± 0.06
Tryptophan, TRP	1.45	1.64	2.06	1.08	0.99	1.44 ± 0.13
Phenylalanine, PHE	0.33	0.50	0.48	0.32	0.27	0.38 ± 0.03
Isoleucine, ILE	0.19	0.27	0.26	0.15	0.15	0.20 ± 0.02
Leucine, LEU	0.37	0.55	0.18	0.20	0.18	0.30 ± 0.05
Lysine, LYS	0.32	0.45	0.26	0.26	0.24	0.30 ± 0.03
Hydroxyproline, HYP	2.74	8.33	2.00	1.28	1.05	3.08 ± 0.91
Proline, PRO	2.20	2.20	1.89	1.97	1.87	2.03 ± 0.07
Total FAAs	33.99 a	70.38 c	53.70 b	28.92 a	31.70 a	43.74 ± 5.34
Essential FAAs	3.44 abc	5.83 d	4.17 bc	2.60 ab	2.39 ab	3.68 ± 0.42

Means followed by the same letter within each row are not significantly different (Tukey's HSD). Values after ± are standard errors (SE). 't' represents trace levels detected and was not included in the quantification.

and essential FAAs. Again, lingonberries examined in this study were comparable to some previously published FAA levels for fruits, including apples, pears, strawberries, etc. described above.

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