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*Market driven authentic non-timber forest products from the Baltic Sea region*

## Quality characterization of bilberries and lingonberries picked from different countries in 2019 and 2020


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# Content

# 1. Introduction

The market of plant-based products is growing and standing out in the competition is becoming more difficult (Hoffman, 2019; von Koeller et al., 2019; New Hope, 2018). There are many fraud products in the market that are competing unfairly with authentic products. The fraud products do not contain the ingredients described in the packaging or they might not originate from the designated place. Sometimes products are adulterated by diluting highly valued ingredients with less valued plants or cultivars (Gardana et al., 2018; Charlebois et al., 2016; Johnson, 2014; Cordella et al., 2002). For example, bilberries (*Vaccinium myrtillus* L.) are diluted with blueberries (*Vaccinium corymbosum* L.), chokeberries (*Aronia*), black soybeans hull (*Glycine max*) and black rice husks (*Oryza sativa*) (Hurkova et al., 2019; Gardana et al., 2018; Primetta et al, 2013; Foster, Blumenthal, 2012). Thus, proving the quality and authenticity is important in the market of plant-based products.

Proving the quality of plant-based products is easier said than done. There are many factors affecting the chemical composition of plant species, such as genetic variations, geographical location and growing conditions (the weather and soil). Also, processing such as freezing, drying, grinding and extracting affects the compounds. Overall, there are many sources that can cause natural variations in the chemical content of plants and plant-based products (Shonte et al., 2020; Karam et al., 2016; Naidu et al., 2016; Kalt et al., 2008; Manach, et al., 2004).

There is lack of uniform and standardized quality verification methods and often the analyses are based on established methods described in the research papers (Kalt et al., 2008; Prior et al., 2005; Manach, et al., 2004). Because of this, it is important to estimate reliability and comparability of the method. Proving quality also requires knowledge of the chemical composition of the plant (Kalt et al., 2008; Prior et al., 2005; Manach et al., 2004).

Polyphenol content and antioxidant capacity are suitable and recommendable analysis methods for all plants (Floegel et al., 2011; Kalt et al., 2008; Ainsworth, Gillespie, 2007; Waterhouse, 2002). However, many species contain compounds of special interest, such as bilberries contain anthocyanins. Thus, also analyses of the compounds of special interest are needed (Primetta et al., 2013; Može et al., 2011; Kähkönen et al., 2003; Jaakola et al., 2002).

Quality verification methods can also be used to prove authenticity. When anthocyanin content of bilberries is measured, the anthocyanin profile can be measured at the same time. If the profile is not typical for bilberries, meaning that all 15 anthocyanins are not found, there are some additional anthocyanins or that the major anthocyanins are not delphinidin or cyanidin glucosides, the sample is unlikely to be pure bilberry or bilberry at all. If the sample doesn't contain anthocyanins, the sample is a clear fraud (Gardana et al., 2018; Primetta et al, 2013; Foster, Blumenthal, 2012). Clear frauds can often be discovered already when analyzing polyphenol content or antioxidant capacity and comparing the results with those of authentic species and products (Sekizawa et al., 2012; Zheng, Wang, 2003).

LAMMC analyzed the quality of bilberries and lingonberries picked from Finland, Norway, Latvia and Lithuania in 2019 and 2020. Centria analyzed the quality of the same berries picked in 2020. The purpose of the study was to determine the quality of the berries and see whether there are differences between the countries and the origin could be identified. The analyses also gathered information on intra-species quality variations, which will allow more comprehensive quality comparison of lingonberry and bilberry samples in the future.

# 2. Quality results of berries obtained by LAMMC

It is important to know to what extend chemical composition of economically important wild berries such as bilberries and lingonberries depends on geographical origin. Therefore, in this study, chemical composition of bilberry samples collected from 3 different locations in Norway, Finland, Latvia and Lithuania were analyzed.

## 2.1 Materials and Methods

### 2.1.1 Samples

The ripe berries of bilberry (*V. myrtillus*) and lingonberry (*V. vitis-idaea*) were handpicked during the summers of 2019 and 2020 at the time periods when they are typically harvested for commercial purposes in Norway, Finland, Latvia and Lithuania in three different locations (Tables 1 and 2).
The berry samples were cooled immediately to below 10 °C then frozen and stored at -18 °C until use.

**Table 1**. Locations of bilberry samples collection in Norway (NOR), Finland (FIN), Latvia (LVA) and Lithuania (LTU).

| No. | Sample | Country code | Coordinates |
|---|---|---|---|
| 1 | B1 | NOR | 69.6944201 / 18.9911423 |
| 2 | B2 | NOR | 69.7512821 / 19.0257227 |
| 3 | B3 | NOR | 69.6708430 / 18.618162 |
| 4 | B4 | FIN | lat: 64° 51.6904', lon: 26° 42.2660' (Puutturi) |
| 5 | B5 | FIN | lat: 64° 59.1702', lon: 25° 54.2195' (Nivalankangas) |
| 6 | B6 | FIN | lat: 65° 13.7528', lon: 25° 33.5924' (Onkamo) |
| 7 | B7 | LVA | 57.142528, 21.865862 Zlekas 1 |
| 8 | B8 | LVA | 57.150996, 21.851492 Zlekas 2 |
| 9 | B9 | LVA | 57.146518, 21.872110 Zlekas 3 |
| 10 | B10 | LTU | 54.1228583 / 24.7169587 |
| 11 | B11 | LTU | 54.7215902 / 23.5088667 |
| 12 | B12 | LTU | 55.0746818 / 22.4706382 |

**Table 2**. Locations of lingonberry samples collection in Norway (NOR), Finland (FIN), Latvia (LVA) and Lithuania (LTU).

| No. | Sample | Country code | Coordinates |
|---|---|---|---|
| 1 | L1 | NOR | 69.7258451/19.1190780 |
| 2 | L2 | NOR | 69.7524024 / 19.0182839 |
| 3 | L3 | NOR | 69.5621356 / 18.6874121 |
| 4 | L4 | FIN | lat: 65° 02.4578', lon: 25° 42.8869' (Savihaju) |
| 5 | L5 | FIN | lat: 65° 00.6398', lon: 26° 05.5423' (Karahka) |
| 6 | L6 | FIN | lat: 64° 54.3698', lon: 25° 44.6767' (Hangaskangas) |
| 7 | L7 | LVA | 56.439955 / 22.812806 |
| 8 | L8 | LVA | 56.440443 / 22.823730 |
| 9 | L9 | LVA | 56.444320 / 22.825480 |
| 10 | L10 | LTU | 54.0864744 / 24.6637269 |
| 11 | L11 | LTU | 54.7564095 / 23.4238457 |
| 12 | L12 | LTU | 55.0848645 / 22.4641838 |

### 2.1.2 Extraction

For determination of anthocyanins, total phenols and antioxidant activity 50 g of defrosted berries were homogenized using Polytron (PT 1200E), then 5 grams of the homogenized sample was extracted with 50 mL of acidified (0.5% HCl) aqueous ethanol solution (70% v/v).

### 2.1.3 Analysis

*Determination of total phenolics content (TPC)*

The total phenolics content of berry extracts was determined using the Folin Ciocalteu method as previously described by Bobinaite et al. (2012). Briefly, the test tubes were filled with 1.0 mL of appropriately diluted extract and mixed with 5.0 mL of Folin-Ciocalteu's phenol reagent diluted in distilled water (1/10, v/v) and 4.0 mL of $Na_2CO_3$ (7.5%). The absorbance of the test solution was read at 765 nm after 60 min incubation in the darkness using Genesys-10 UV/Vis spectrophotometer (Thermo Spectronic, Rochester, USA). Gallic acid was used as the standard for the calibration curve, and results were expressed in mg of gallic acid equivalents in 100 g of berries (fw).

*HPLC analysis of anthocyanins*

Anthocyanins were separated using Waters 2695 series HPLC system, equipped with the Waters 2998 photo diode array detector (DAD) (Waters Corporation, USA). Analytical separation was carried out using a LiChroCART Purospher® STAR RP-18 endcapped column (250 × 4.6 mm, 5 µm particle size) with a guard column Purospher STAR RP 18e 4.0 × 4.0 mm 5 µm (Merck KgaA, Germany) using slightly modified procedure of Lätti et al. (2008). The temperature of the column oven was set at 25 °C. The

mobile phase consisted of aqueous 10 % formic acid (eluent A) and ACN–MeOH (85:15, v/v) (eluent B). The gradient program was as follows: 0-2 min 4-6 % eluent B; 2-4 min 6-8 % eluent B; 4-12 min 8-9 % eluent B; 12-46 min 9-11 % eluent B; 46-48 min 11-24 % eluent B; 48-52 min 24-34 % eluent B; 52-59 min 34-80 % eluent B; 59-61 min 80-20 % eluent B; 61-65 min 4 % eluent B. The injection volume was 10 µL.

Anthocyanins were detected at the wavelength of 520 nm. DAD data were recorded from 200 to 600 nm. Anthocyanins in bilberry and lingonberry extracts were identified according to the HPLC retention times (RT) and and UV absorbance maximum, in comparison with commercial standards or with literature data (Lätti et al. 2008).

Commercial standard (cyanidin-3-glucoside) was dissolved in solvent B (10 %) and solvent A (90 %) to generate seven-point external standard calibration curve (concentration range was from 1 to 100 mg/L), whose linearity was acceptable ($R^2$=0.999).

The total content of anthocyanins in the extracts was determined as the sum of the amount of the individually quantified compounds as equivalents of cyanidin-3-glucoside ($C_3G$) per 100g of fw of berry.

*Determination of ferric reducing antioxidant power (FRAP)*

FRAP assay was performed according to the method of Benzie and Strain (1996), with slight modifications (Bobinaitė et al., 2015).

For FRAP assay 0.3 M sodium acetate buffer (pH 3.6) was prepared by dissolving 3.1 g of sodium acetate and 16 mL of acetic acid in 1000 mL of distilled water; 10 mM TPTZ solution was prepared by dissolving 0.031 g TPTZ in 10 mL of 40 mM HCl; 20 mM ferric solution was prepared by dissolving 0.054 g of $FeCl_3 \cdot 6H_2O$ in 10 ml of distilled water. Working FRAP reagent was prepared by freshly mixing acetate buffer, TPTZ and ferric solutions at a ratio of 10:1:1.

For the analysis, 2 mL of freshly prepared FRAP working solution and 20 µL of diluted extract were mixed and incubated for 30 minutes at ambient temperature. The change in absorbance due to the reduction of ferric-tripyridyltriazine (Fe III-TPTZ) complex by the antioxidants present in the samples was monitored at 593 nm using a Genesys-10 UV/Vis (Thermo Spectronic, Rochester, USA) spectrophotometer. The absorptions of blank samples (by applying the same analysis conditions) were tested each time before and after analysis.

Trolox was used as the standard, and the antioxidant activity was expressed as µmol of trolox equivalents (µmol TE) per g of berries (fw).

*Determination of ABTS radical scavenging activity (ABTS RSA)*

The RSA of extracts was also measured by $ABTS^{\bullet+}$ radical cation assay (Re et al., 1999). ABTS solution (2 mM) was prepared by dissolving 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt in 50 mL of phosphate buffered saline (PBS) obtained by dissolving 8.18 g NaCl, 0.27 g $KH_2PO_4$, 1.42 g $Na_2HPO_4$ and 0.15 g KCl in 1 L of pure water. The pH of prepared solution was adjusted to 7.4 using NaOH. Then $K_2S_2O_8$ solution (70 mM) was prepared in pure water.

Working solution ($ABTS^{\bullet+}$ radical cation) was produced by reacting 50 mL of ABTS solution with 200 µL of $K_2S_2O_8$ solution and allowing the mixture to stand in the dark at room temperature for 15–16 h before use.

For the assessment of antiradical activity of the extracts, 2 mL of $ABTS^{\bullet+}$ solution were mixed with 20 µL extract in a 1 cm path length cuvette. The reaction mixture was kept at room temperature in the dark for 30 min, and the absorbance was read at 734 nm.

Trolox was used as the standard, and *ABTS RSA* was expressed as µmol of trolox equivalents (µmol TE) per g of berries (fw).

*Determination of dry matter (DM), total soluble solids (TSS) and pH*
Dry matter content was determined after forced air convention drying at 105 °C to a constant weight. The total soluble solids were determined using a digital refractometer (ATAGO PR-32, Atago Co., Ltd., Tokyo, Japan). The pH was measured using an inoLab Level 1 pH meter with SenTix 81 (WTW) electrode.

**Table 3**. Summary of analysis methods.

| Analysis method | Assay | Unit |
|---|---|---|
| **Polyphenol content (TPC)** | Folin-Ciocalteu | mg GAE/100g fw |
| **Total anthocyanin content (TAC)** | Liquid chromatography | mg $C_3G$/100 g fw |
| **Anthocyanin profile (AP)** | Liquid chromatography | Chromatogram |
| **Antioxidant capacity** | FRAP, ABTS | µmol TE/g fw |
| **Total soluble solids (TSS)** | Refractometric | Brix° |
| **pH** | Potentiometric | - |

## 2.2 Results and discussion

*The pH and soluble solids (SS) content*
The pH measures the acidity and soluble solids shows high positive correlation with sugars content of fruits and berries (Viljakainen et al., 2002). The organoleptic quality and storage life of berries is related to its SS content and acidity (Retamales, Hancock, 2012).

*Bilberries*. The pH values of investigated bilberries varied from 2.94 to 3.47 (Table 4). In 2019, lower mean pH values had berries from LVA and NOR (3.30 and 3.32, respectively), whereas in 2020, the lowest mean pH was measured in bilberries from LTU (2.95). In 2020, the pH of bilberries from all countries was significantly lower than in 2019 (Table 4).
Previously, Giovanelli and Buratti (2009) reported that pH of Italian bilberries ranged from 3.13 to 3.22. Turkben et al. (2008) reported pH values between 2.77 and 2.95 among wild bilberries from western Turkey. These results are in accordance to the pH values estimated in our study.
The content of SS in bilberries varied from 9.4 to 15.8 Brix**°** (Table 5). In 2019, berries from NOR and FIN had higher mean SS content (12.6 and 13.0 Brix**°**, respectively) than berries from LVA and LTU, whereas in 2020, the mean SS content of berries from all countries was similar (Table 5). In 2020, SS values of the bilberry samples, with exception of the ones collected in LTU location B10, were significantly lower than respective values determined in 2019.

**Table 4.** pH values of bilberries.

| Country code | Collection location | pH | |
|---|---|---|---|
| | | 2019 | 2020 |
| NOR | B1 | 3.34 ± 0.001bc* | 3.11 ± 0.025b |
| | B2 | 3.34 ± 0.020bc* | 3.00± 0.006cd |

| | | | |
|---|---|---|---|
| | B3 | 3.28 ± 0.021cd* | 3.02 ± 0.020c |
| | Mean | 3.32 ± 0.033b* | 3.04± 0.051b |
| **FIN** | B4 | 3.27 ± 0.030d* | 3.27 ± 0.012a |
| | B5 | 3.34 ± 0.021bc* | 3.10 ± 0.006b |
| | B6 | 3.46 ± 0.010a* | 3.11 ± 0.021b |
| | Mean | 3.36 ± 0.085ab* | 3.16 ± 0.080a |
| **LVA** | B7 | 3.28 ± 0.020 cd* | 3.00 ± 0.010cd |
| | B8 | 3.35 ± 0.040b* | 3.10 ± 0.010b |
| | B9 | 3.27 ± 0.020d* | 3.00 ± 0.010cd |
| | Mean | 3.30 ± 0.045b* | 3.03 ± 0.051b |
| **LTU** | B10 | 3.44 ± 0.030a* | 2.94 ± 0.012e |
| | B11 | 3.32 ± 0.030bcd* | 2.96 ± 0.021de |
| | B12 | 3.47 ± 0.001a* | 2.95 ± 0.010e |
| | Mean | 3.41 ± 0.072a* | 2.95± 0.016c |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (B1-12) ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).*

SS values of investigated bilberries were in accordance with previously reported findings. Turkben et al. (2008) reported that the SS content in *V. myrtillus* berries from Turkey was from 9.0 to 11.0%. The SS content in wild bilberries from Italy varied from 10.8 to 11.1% (Giovanelli, Buratti, 2009), whereas SS content in bilberries from Romania - from 9.2 to 13.7% (Oancea et al., 2013). SS content in Polish bilberries was 13.0% (Ochmian et al., 2009).

**Table 5.** Soluble solids (SS) content of bilberries.

| **Country code** | **Collection location** | **SS, Brix°** | |
|---|---|---|---|
| | | **2019** | **2020** |
| **NOR** | B1 | 12.2 ± 0.01ef* | 9.5 ± 0.20e |
| | B2 | 12.9 ± 0.10bc* | 10.5 ± 0.07b |
| | B3 | 12.6 ± 0.12cd* | 10.0 ± 0.16cd |
| | Mean | 12.6 ± 0.31ab* | 10.0 ± 0.45a |
| **FIN** | B4 | 10.2 ± 0.09j* | 9.7 ± 0.06de |
| | B5 | 13.0 ± 0.15b* | 10.3 ± 0.12bc |
| | B6 | 15.8 ± 0.14a* | 9.6 ± 0.10e |
| | Mean | 13.0 ± 0.43a* | 9.9 ± 0.34a |
| **LVA** | B7 | 10.9 ± 0.17i* | 10.2 ± 0.21bc |

| | | | |
|---|---|---|---|
| | B8 | 11.7 ± 0.09gh* | 10.2 ± 0.05bc |
| | B9 | 11.5 ± 0.16h* | 10.1 ± 0.07c |
| | Mean | 11.4 ± 0.38b* | 10.2 ± 0.12a |
| **LTU** | B10 | 11.9 ± 0.06fg | 12.1 ± 014a |
| | B11 | 12.0 ± 0.10fg* | 9.4 ± 0.15e |
| | B12 | 12.4 ± 0.05de* | 9.5 ± 0.10e |
| | Mean | 12.1 ± 0.24ab* | 10.3 ± 0.33a |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (B1-12) ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).*

*Lingonberries.* The pH of lingonberries varied from 2.66 to 3.03 and was lower than that of bilberries (Tables 4 and 6). In 2019, there were no significant differences between the mean pH values of berries collected in different countries, whereas in 2020 lingonberries from NOR had significantly higher mean pH value (Table 6). Similarly, as observed with bilberries, in 2020, the pH values of lingonberries collected in all countries (NOR, FIN, LVA and LTU) were significantly lower. The pH values of lingonberries measured in this study are similar to what was previously reported (2.74-2.90) (Lee, Finn, 2012).

Different growth and environment conditions such as temperature, day length, light intensity, possibly influence the SS of the berries (Primetta et al., 2013). The SS content of investigated lingonberries varied from 10.4 to 15.3 Brix° (Table 7). In 2019, lingonberries growing in LTU and FIN contained higher levels of SS, whereas in 2020, higher SS content had berries from LTU and LVA (Table 7). In 2020, the lowest mean SS content (10.9 Brix°) was determined in lingonberries from NOR. Similarly, to that observed in bilberries, lingonberries from NOR, FIN and LTU (L11 and L12) had significantly lower SS content in 2020 than in 2019.

**Table 6.** pH values of lingonberries.

| Country code | Collection location | pH | |
|---|---|---|---|
| | | **2019** | **2020** |
| **NOR** | L1 | 3.03 ± 0.040a* | 2.87 ± 0.015a |
| | L2 | 2.93 ± 0.030bcde* | 2.87 ± 0.010a |
| | L3 | 2.97 ± 0.010abcd* | 2.81 ± 0.015b |
| | Mean | 2.98 ± 0.050a* | 2.85 ± 0.033a |
| **FIN** | L4 | 2.98 ± 0.040abc* | 2.80 ± 0.020b |
| | L5 | 2.93 ± 0.010bcde* | 2.73 ± 0.006d |
| | L6 | 2.93 ± 0.030a* | 2.73 ± 0.006d |
| | Mean | 2.98 ±0.054a* | 2.75 ± 0.038b |
| **LVA** | L7 | 3.04 ± 0.050abcd* | 2.74 ± 0.015cd |

| | | | |
|---|---|---|---|
| | L8 | 2.97 ± 0.040de* | 2.75 ± 0.015cd |
| | L9 | 2.88 ± 0.020de* | 2.74 ± 0.015cd |
| | Mean | 2.91 ± 0.056a* | 2.75 ± 0.014b |
| **LTU** | L10 | 2.89 ± 0.001cde* | 2.78 ± 0.020bc |
| | L11 | 2.87 ± 0.050e* | 2.78 ± 0.021bc |
| | L12 | 3.01 ± 0.001ab* | 2.66 ± 0.006e |
| | Mean | 2.92 ± 0.070a* | 2.74 ± 0.059b |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (L1-12) (p < 0.05). Significant differences between 2019 and 2020 are indicated by asterisks (*) (p < 0.05).*

**Table 7.** Soluble solids content (SS) content of lingonberries.

| Country code | Collection location | SS, Brix° | |
|---|---|---|---|
| | | 2019 | 2020 |
| **NOR** | L1 | 12.1 ± 0.04f* | 10.4 ± 0.06f |
| | L2 | 13.6 ± 0.05d* | 11.5 ± 0.06d |
| | L3 | 13.6 ± 0.1d* | 10.7 ± 0.10e |
| | Mean | 13.1 ± 0.75b* | 10.9 ± 0.50c |
| **FIN** | L4 | 13.8 ± 0.05d* | 12.8 ± 0.12c |
| | L5 | 14.7 ± 0.11b* | 12.8 ± 0.06c |
| | L6 | 15.1 ± 0.12a* | 11.6 ± 0.05d |
| | Mean | 14.5 ± 0.58a* | 12.4 ± 0.57b |
| **LVA** | L7 | 12.5 ± 0.09e | 13.2 ± 0.15b* |
| | L8 | 12.4 ± 0.06e | 13.4 ± 0.06b* |
| | L9 | 12.3 ± 0.14ef | 13.3 ± 0.12b* |
| | Mean | 12.4 ± 0.12b | 13.3 ± 0.13a* |
| **LTU** | L10 | 14.2 ± 0.08c | 14.1 ± 0.06a |
| | L11 | 15.3 ± 0.12a* | 13.9 ± 0.08a |
| | L12 | 15.3 ± 0.06a* | 13.2 ± 0.06b |
| | Mean | 14.9 ± 0.56a* | 13.7 ± 0.41a |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (L1-12) (p < 0.05). Significant differences between 2019 and 2020 are indicated by asterisks (*) (p < 0.05).*

*Total phenolics content (TPC)*

*Bilberries.* The total phenolics content (TPC) of the tested bilberry samples ranged from 452 (LTU B12 in 2020) to 902 mg/100 g fw (NOR B2 in 2019) (Table 8). The highest average TPC value was found in the berries collected in Norway (791 mg/100 g fw in 2019 and 660 mg/100 g fw in 2020). Both years (in 2019 and 2020), the lowest mean TPC value was found in berry samples from Lithuania (587 mg/100 g fw in 2019 and 546 mg/100 g).

Total phenolics content of investigated samples were in accordance with previously reported findings. For instance, TPC value of bilberries collected from natural population in Macedonia was 706 mg/100 g fw (Stanoeva et al., 2017) and in bilberries from Serbia - 890 mg/100 g fw (Šavikin et al., 2009). However, Milivojević et al., (2013) determined somewhat lower TPC of bilberries collected in Serbia (387 mg/100 g fw). The TPC of bilberries from the forest of Poland was reported to be 640 mg/100 g fw (Ochmian et al., 2009). The TPC values of bilberries from natural populations in Norway were reported to range between 512 and 674 mg/100 g fw (Skrede et al., 2012; Rohloff et al., 2015).

**Table 8.** Total phenolics content (TPC) of bilberries mg/ 100 g fw.

| Country code | Collection location | TPC, mg/100g fw | |
|---|---|---|---|
| | | 2019 | 2020 |
| NOR | B1 | 697 ± 29.9c* | 593 ± 30.1cde |
| | B2 | 902 ± 24.5a* | 713 ± 29.1a |
| | B3 | 776 ± 35.0b* | 675 ±32.7ab |
| | Mean | 791 ± 28.8a* | 660 ± 31.6a |
| FIN | B4 | 553 ± 26.1ef | 537 ± 24.6e |
| | B5 | 586 ± 22.4def | 549 ± 28.1e |
| | B6 | 700 ± 29.8c* | 572 ± 26.6e |
| | Mean | 613 ± 24.6b* | 553 ± 26.5b |
| LVA | B7 | 629 ± 13.9cd | 650 ± 15.3abcd |
| | B8 | 629 ± 18.1cd | 649 ± 10.9abcd |
| | B9 | 587 ± 19.6def | 654 ± 20.8abc* |
| | Mean | 615 ± 17.0b | 651 ± 15.7a* |
| LTU | B10 | 534 ± 24.3f | 579 ± 20.9de |
| | B11 | 607 ± 26.2def | 607 ± 30.7bcde |
| | B12 | 620 ± 21.4de* | 452 ± 19.2f |
| | Mean | 587 ± 29.4b | 546 ± 21.9b |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (B1-12) ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).*

It has previously been shown that both the growing conditions and the genetic origin of the wild bilberries affects the content of phenolic compounds (Uleberg et al., 2012; Mikulic-Petkovsek et al.,

2015). The latitude-related factor was reported as having high influence on the quality and quantity of phenolic compounds in bilberries; suggesting that higher phenolic contents may be supported by northern latitudes, altitude, and sunny weather (Ștefănescu et al., 2020). In previous studies, higher contents of phenolic compounds and anthocyanins were detected in the bilberry clones originating from higher latitudes (Lätti et al., 2008; Åkerström et al., 2010; Uleberg et al., 2012). Interestingly, our data also shows that the mean TPC of berries from the Norway (the most northern country covered in the study) was the highest, whereas the mean TPC of samples from the most southern country (Lithuania) was the lowest (Table 8). Furthermore, bilberry samples from Norway collected in the northernmost location (B2) had the highest TPC value, whereas TPC value of the samples collected in the southernmost location (B1) in 2019 and 2020 was by 23 and 17% lower, respectively. Similarly, among samples collected in Finland, the highest TPC value (700 mg/100g fw in 2019 and 572 mg/100g fw in 2020) was determined in berries from the northernmost location (B6) (Table 8). In 2019 the same trend could also be observed for the bilberry samples from Lithuania, where TPC values also slightly increased with higher latitudes. On other hand, for Lithuanian samples this trend was not observed in 2020. The samples collected in Latvia had very similar TPC, most likely due to the proximity of the sample collection sites.

Our results also indicate that there were significant yearly variations in the TPC values of berries (Table 8), suggesting that although genotype affects the TPC in bilberries, its final content also depends on weather conditions.

*Lingonberries.* Total phenolics content in lingonberry samples were within similar range as in bilberries and varied from 477 (NOR L2 in 2020) to 776 mg/100 g fw (NOR L3 in 2019) (Table 9).

**Table 9.** Total phenolics content (TPC) of lingonberries mg/ 100 g fw.

| Country code | Collection location | TPC, mg/100g fw | |
|---|---|---|---|
| | | 2019 | 2020 |
| NOR | L1 | 587 ± 30.5ef | 688 ± 24.4a* |
| | L2 | 725 ± 30.7abc* | 477 ± 21.8d |
| | L3 | 776 ± 21.9a* | 567 ± 25.3b |
| | Mean | 696 ± 27.7a* | 577 ± 23.9a |
| FIN | L4 | 722 ± 39.1abc* | 481 ± 20.1d |
| | L5 | 698 ± 17.1abcd* | 566 ± 18.3b |
| | L6 | 733 ± 29.0ab* | 559 ± 28.8bc |
| | Mean | 718 ± 28.4a* | 536 ± 22.4a |
| LVA | L7 | 667 ± 30.1bcde* | 528 ± 16.4bcd |
| | L8 | 678 ± 26.1bcd* | 538 ± 10.9bcd |
| | L9 | 662 ± 34.2bcde* | 521 ± 20.0bcd |
| | Mean | 669 ± 30.1ab* | 529 ± 15.8a |
| LTU | L10 | 630 ± 32.5def | 637 ± 30.4a |

| | | |
|---|---|---|
| L11 | 579 ± 20.2f | 571 ± 13.7b |
| L12 | 640 ± 35.7cdef | 497 ± 23.6cd* |
| Mean | 617 ± 29.5b | 568 ± 22.6a* |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (L1-12) ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).*

Previously proposed classification of fruits based on their TPC distinguishing between low (<100 mg /100 g), medium (100-500 mg/100 g) and high (>500 mg/100 g) values, indicates that investigated berries are a good source of these compounds (Vasco et al., 2008).

Total phenolics content of investigated lingonberry samples was in accordance with previously reported findings. For instance, TPC of lingonberries grown in the central region of Poland ranged from 468 to 661 mg/100 g fw (Dróżdż et al., 2018). Similar values (ranging from 431to 660 mg/100 g fw) were measured in lingonberry cultivars grown in Oregon (United States) (Lee, Finn, 2012). Higher TPC values (ranging from 714-791 mg/100 g fw) were reported in lingonberries growing in natural habitats in Bulgaria (Dincheva, Badjakov, 2016).

In the year 2019, lingonberry samples with the highest TPC values were collected in the northern countries (NOR and FIN), however this trend was not observed in 2020, where the average TPC values were similar for the samples collected in all four countries covered in the study (Table 9). Furthermore, in 2020 the average TPC values of lingonberries from NOR, FIN and LVA were significantly lower than respective values determined in 2019. In 2020, the average TPC values of lingonberry samples were up to 25% lower, which also suggest significant impact of weather conditions on the accumulation of phenolic compounds.

*Total anthocyanins content (TAC)*

*Bilberries.* Bilberry is one of the riches sources of anthocyanins that have multiple biological activities (Khoo et al., 2017).

The mean TAC value of investigated bilberry samples was 401.9 and 327.5 mg/100 g fw in 2019 and 2020, respectively. It is worth noting that within the same year the mean TAC values of the berry samples from different countries did not differ significantly, the only exception - Lithuanian bilberries in 2019, that had significantly lower mean TAC (Table 10). The highest TAC had two berry samples collected in 2019 in the northernmost locations (B2 and B6) in Norway and Finland (475.4 and 454.6 mg/100 g fw, respectively).

TAC values of investigated bilberry samples were in accordance with previously reported findings. For instance, Skrede and co-workers (2012) reported that the concentration of anthocyanins in bilberry samples was from 429 to 627 mg/100 g fw (Skrede et al., 2012). TAC of bilberries from Macedonia was 507 mg/100 g fw (Stanoeva et al., 2017). Rohloff et al., (2015) reported somewhat lower amounts of total anthocyanins in Norwegian bilberries (from 330 to 449 mg/100 g fw) (Rohloff et al., 2015), whereas TAC of Finish bilberries from 20 different populations varied from 350 to 525 mg/100 g fw (Lätti et al. 2008).

It has been shown that genotype and environment interaction affect accumulation of anthocyanins in bilberries (Zoratti et al., 2015; Rohloff et al., 2015; Mikulic-Petkovsek et al., 2015). The increasing trend in anthocyanin content has been repeatedly observed in bilberries toward northern latitudes of Europe (Lätti et al., 2008; Åkerström et al., 2010). However, when effects of different environmental

factors on berry chemical composition was studied in eight forest fields of bilberry in Northern-, Mid- and Southern Norway previous findings concerning latitudinal effects on anthocyanin concentration were not confirmed (Rohloff et al. 2015). The authors concluded that most probably the environmental impacts confounded the genetic (population) effects (Rohloff et al. 2015).
With regard to increased TAC towards northern latitudes the trend was not clear in the present study. In 2019, the highest TAC had two samples collected in the northernmost locations (B2 and B6), however in 2020, the berries from locations B2 and B6 had high, but not the highest TAC (Table 10).

**Table 10.** Total anthocyanins content (TAC) of bilberries mg/100 g fw.

| Country code | Collection location | mg 100 g FW | |
|---|---|---|---|
| | | 2019 | 2020 |
| NOR | B1 | 363.2 ± 10.55 cd* | 302.8 ± 13.32 bc |
| | B2 | 475.4 ± 14.50 a* | 356.3 ± 10.04 a |
| | B3 | 420.4 ± 15.72 b* | 330.6 ± 16.78 ab |
| | Mean | 419.7 ± 50,03 a* | 329.9 ± 26.02 a |
| FIN | B4 | 371.1 ± 7.24 c* | 296.8 ± 9.03 c |
| | B5 | 380.0 ± 10.41 c* | 341.2 ± 6.97 a |
| | B6 | 454.6 ± 16.75 ab* | 353.8 ± 10.26 a |
| | Mean | 401.6 ± 40.6 ab* | 330.6 ± 27.04 a |
| LVA | B7 | 424.2 ± 8.48 b* | 341.3 ± 7.19 a |
| | B8 | 427.4 ± 10.34 b* | 332.0 ± 6.80 ab |
| | B9 | 420.9 ± 9.77 b* | 340.2 ± 7.99 a |
| | Mean | 424.2 ± 8.75 a* | 337.8 ± 7.73 a |
| LTU | B10 | 329.9 ± 7.75 d | 340.8 ± 15.01 a |
| | B11 | 373.0 ± 11.17 c | 360.9 ± 11.12 a |
| | B12 | 383.9 ± 9.26 c* | 232.7 ± 10.8 d |
| | Mean | 362.3 ± 26.06 b* | 311.5 ± 60.68 a |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (L1-12) ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).*

In 2020, TAC of the bilberry samples, with exception of the ones collected in LTU location B10, were from 3 to 39% lower than respective values determined in 2019, which suggest significant influence of environmental factors on the accumulation of anthocyanins.

Using HPLC-DAD, 15 anthocyanins were identified in bilberries based on their retention times UV/Vis spectra compared with standards and published data (Figure 1).
The contents of the anthocyanin glycosides were significantly different between the countries. In

bilberries from NOR and FIN the major anthocyanin was Dp-3-ara (13.7-16.0% of total anthocyanins), followed by Dp-3-gal (11.6-14.0% of total anthocyanins) and Dp-3-glc (11.0-13.6% of total anthocyanins). In bilberries from LTV and LTU the major anthocyanin was Cy-3-glc (12.9-14.4% of total anthocyanins), followed by Cy-3-gal (10.5-14.0% of total anthocyanins) and Dp-3-glc (10.8-12.7% of total anthocyanins) (Figure 2A and B). Both years of investigation bilberries from LTV and LTU had significantly lower contents of Dp-3-gal and Dp-3-glc than berries from NOR and FIN, but higher contents of Pn-3-glc (Figure 2A and B).

The total average proportion of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv) glycosides in bilberries was 35.59%, 32.79%, 14.28%, 6.34%, and 11.01 %, respectively (Figure 3A and B). In this study the average proportions of Dp, Cy, Pt, Pn, and Mv were similar to those previously reported in bilberries (Lätti et al., 2008).

The contents of Dp were significantly higher in the bilberries from NOR and FIN (41.1 and 38.7% in 2019 and 2020, respectively) than in berries from LTV and LTU (30.6 and 31.9% in 2019 and 2020, respectively), whereas the content of Cy were significantly higher in the berries from LTV and LTU (37.5 and 33.6% in 2019 and 2020, respectively), than in bilberries from NOR and FIN (30.3 and 29.7% in 2019 and 2020, respectively) (Figure 3A and B).

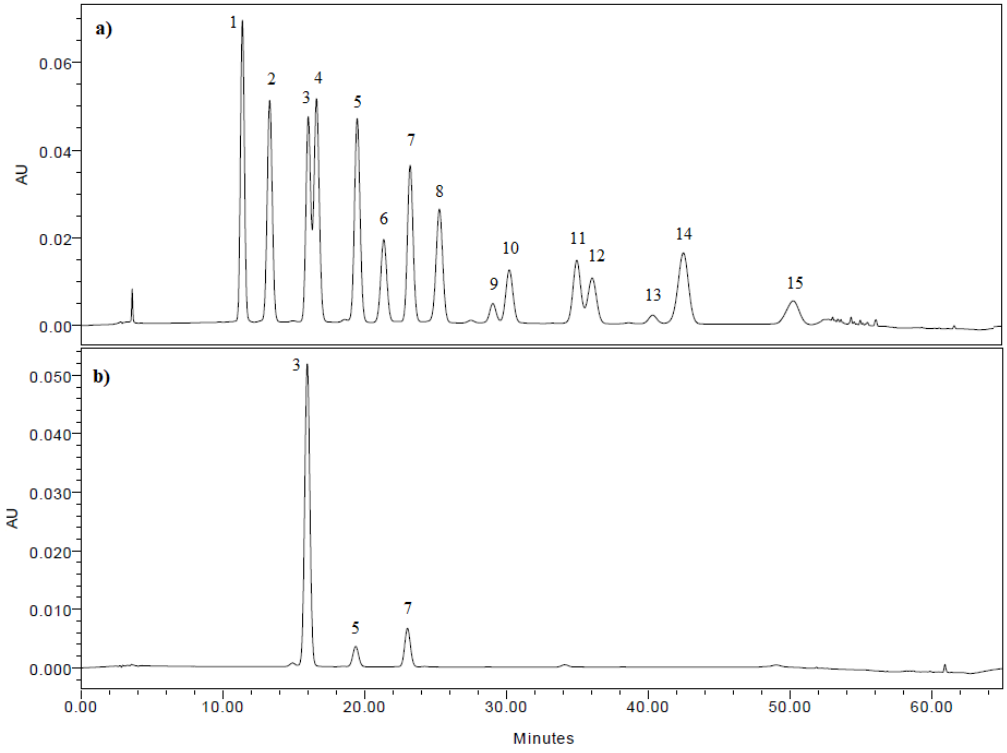


**Figure 1.** HPLC-DAD profiles of anthocyanins in bilberries (a) and lingonberries (b) at 520 nm. Peak identification: *1. Delphinidin-3-galactoside; 2. Delphinidin-3-glucoside; 3. Cyanidin-3-galactoside; 4. Delphinidin-3-arabinoside; 5. Cyanidin-3-glucoside; 6. Petunidin-3-galactoside; 7. Cyanidin-3-arabinoside; 8. Petunidin-3-glucoside; 9. Peonidin-3-galactoside; 10. Petunidin-3-arabinoside; 11. Peonidin-3-glucoside; 12. Malvidin-3-galactoside; 13. Peonidin-3-arabinoside; 14. Malvidin-3-glucoside; 15. Malvidin-3-arabinodside.*

Furthermore, higher average proportion of Pn was detected in Latvian and Lithuanian bilberries (varied from 6.9 and 8.9%) than in Norwegian and Finish bilberries (varied from 4.4-5.4%) (Figure 3A

and B). Lätti et al. (2008) also reported that the content of Pn was significantly lower in northern bilberries compared to those gathered in the south.

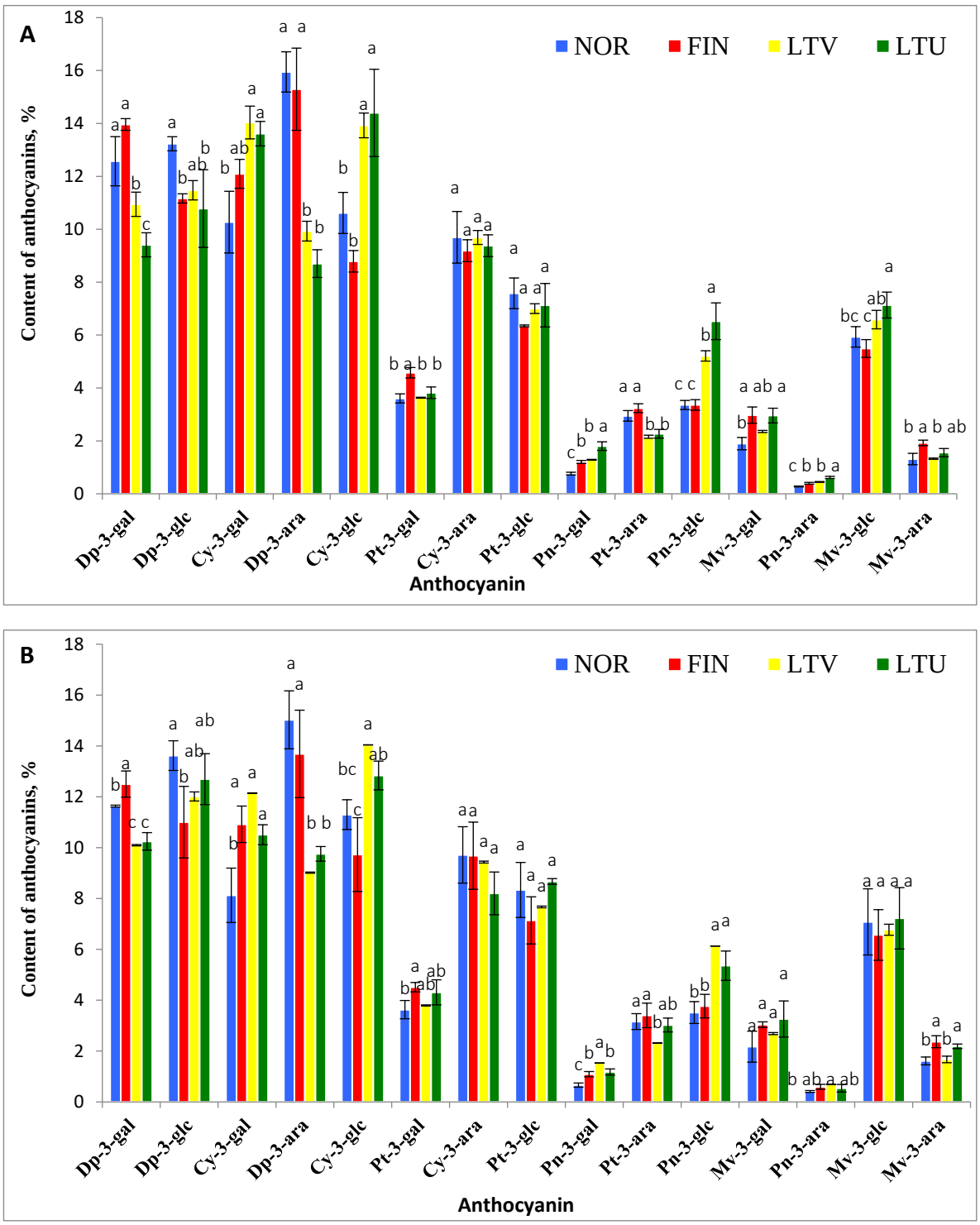


**Figure 2.** Contents of individual anthocyanins in bilberry samples (%) harvested in 2019 (A) and 2020 (B). Different letters above the bars of each anthocyanin indicate significant differences between the mean values ($p < 0.05$).

In the study reported by Martinelli et al. (1986) the Cy glycosides were more abundant in bilberries from northern latitudes (Norway, Sweden) compared to more southern regions (Italy, Romania,

Poland), which agrees to our findings. In addition, Lätti et al. (2008) also found that delphinidin glycosides dominated in bilberries from northern regions whereas Cy glycosides were most common in southern regions of Finland. A positive effect of low temperatures on levels of delphinidin glycosides in bilberries was also reported by Uleberg et al. (2012). On other hand, the differences in the proportions of Dp and Cy between berries from southern and northern regions might also be of genetic origin, since Castellarin et al. (2006) determined that the ratio between delphinidin and cyanidin is largely under genetic control.

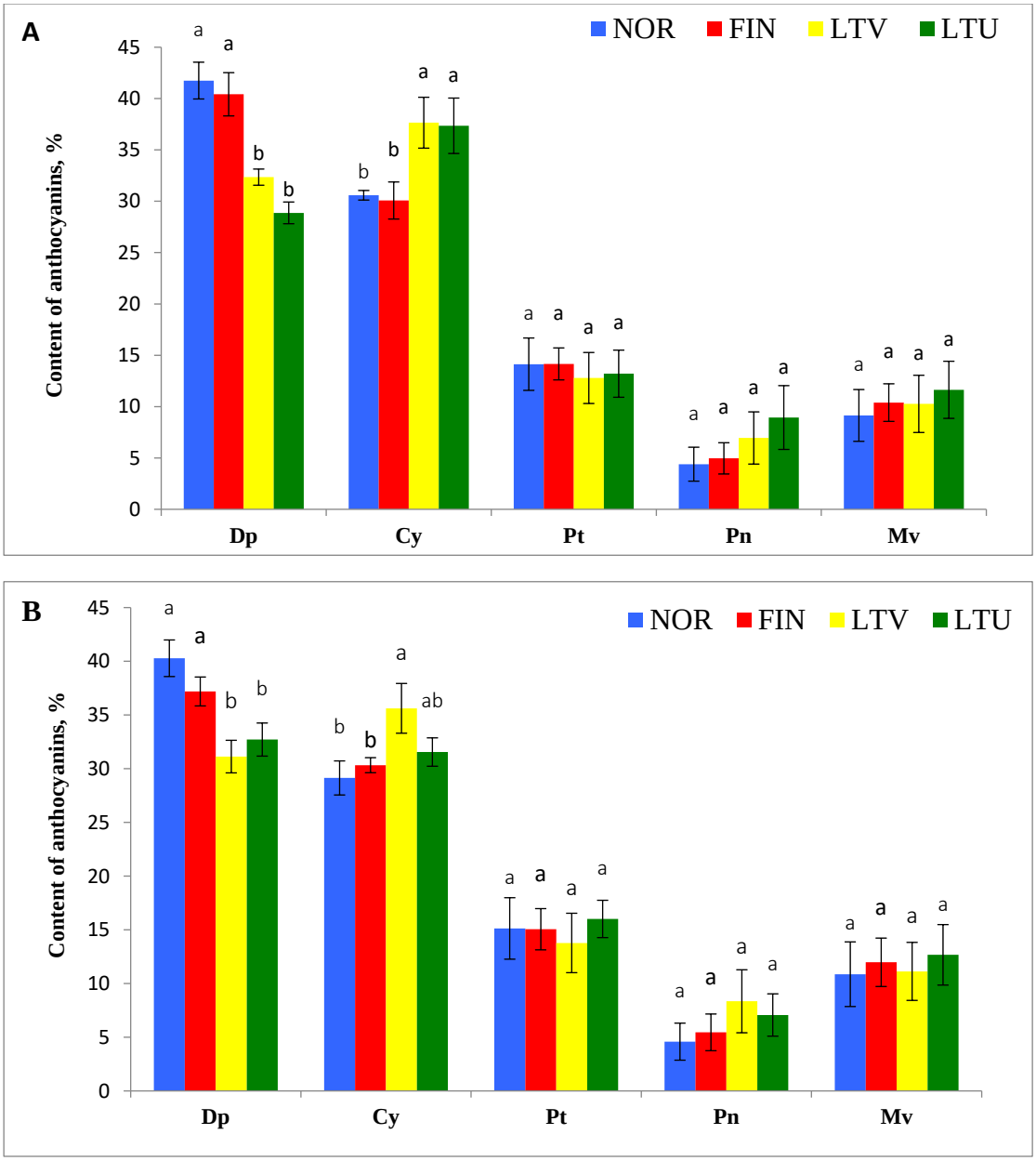


**Figure 3.** Contents of anthocyanins in bilberry samples (%) harvested in 2019 (A) and 2020 (B). Statistically significant differences ($p < 0.05$) are marked with different letters.

*Lingonberries.* For the total anthocyanin contents, the lingonberry samples presented significantly lower values (19.8 - 57.0 mg/100 g fw) in comparison to the bilberries (Tables 10 and 11). As reported in the literature, the main flavonoids in lingonberries are not anthocyanins but flavan-3-ols (catechin

and epicatechin) as well as flavonols, mainly quercetin glycosides (Ieri et al., 2013; Hajazimi et al., 2016).

Previously reported total anthocyanins content (determined by HPLC) in lingonberries grown in the research plot in Cornavallis (USA) ranged from 27.4 to 51.6 mg/100 g fw (Lee, Finn, 2012) and in lingonberries harvested in the forests of Poland - from 32 to 47 mg/ 100g fw (Dróżdż et al., 2017). These results are similar to TAC values determined in our study. Somewhat higher total anthocyanins content was previously reported in lingonberries from Finland (77.5 mg/100 g fw) (Koponen et al., 2007). TAC (measured by pH differential method) in lingonberry wild clones and cultivars grown in Canada varied from 12.1 to 85.5 mg/100 g fw (Debnath, Sion, 2009).

**Table 11.** Total anthocyanins content (TAC) of lingonberries mg/ 100 g fw.

| Country code | Collection location | TAC, mg/100g fw | |
|---|---|---|---|
| | | 2019 | 2020 |
| NOR | L1 | 49.2 ± 2.61c | 56.0 ± 2.10ab* |
| | L2 | 57.0 ± 2.05a* | 43.7 ± 1.19c |
| | L3 | 45.7 ± 2.00cd | 53.6 ± 2.18ab* |
| | Mean | 50.6 ± 2.22a | 51.1 ± 1.82a |
| FIN | L4 | 54.3 ± 1.73ab | 53.4 ± 2.00ab |
| | L5 | 41.7 ± 1.84d | 56.1 ± 2.18a* |
| | L6 | 49.9 ± 0.59bc | 51.7 ± 1.50b |
| | Mean | 48.6 ± 1.39a | 53.7 ± 1.89a* |
| LVA | L7 | 30.4 ± 1.25ef | 31.1 ± 1.05de |
| | L8 | 31.3 ± 1.75e | 32.0 ± 1.00d |
| | L9 | 29.4 ± 1.10efg | 32.9 ± 0.64d |
| | Mean | 30.4 ± 1.33b | 32.0 ± 0.88b |
| LTU | L10 | 25.4 ± 1.01g | 26.2 ± 1.11f |
| | L11 | 20.4 ± 0.90h | 19.8 ± 0.90g |
| | L12 | 26.5 ± 1.32fg | 27.4 ± 1.17ef |
| | Mean | 24.1 ± 1.08c | 24.5 ± 1.06c |

*Note. Values are presented as means ± standard deviation. Different letters within the same column indicate significant differences between the collection locations (L1-12) ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).*

Lingonberry samples from NOR and FIN both years (2019 and 2020) had the highest TAC (varied from 41.7 to 57.0 mg/100 g fw), followed by berry samples from LVA (varied from 29.4 to 32.9 mg/100 g fw) and LTU (varied from19.8 to 27.4 mg/100 g fw) (Table 11). In this study, significant annual variation in TAC of lingonberry samples collected from few locations (L1, L2, L3 and L5) was observed.

Using HPLC-DAD, three anthocyanins (cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin-3-glucoside) were identified in lingonberries (Figure 1). Lingonberry samples contained only cyanidin-based anthocyanins as was previously reported by other researchers (Kähkönen et al., 2003; Ek et al., 2006; Lehtonen et al., 2009; Lätti et al., 2011). Cyanidin-3-galactoside, comprising from 74.4 to 83.5% of total anthocyanins present, was the dominant anthocyanin, as similarly reported in other studies (Bakowska-Barczak et al., 2007; Ek et al., 2006; Kähkönen et al., 2003; Lätti et al., 2011; Lee, Finn, 2012).

Cyanidin-3-arabinoside made up 12.2-18.6% and cyanidin-3-glucoside - 4.3-9.7% of the total anthocyanins in the investigated lingonberries. The mean percentage values of the individual anthocyanins in lingonberries harvested in different counties in 2019 and 2020 are shown in Figure 4A and B. Similar distribution of anthocyanins in lingonberries (cyanidin-3-galactoside (84%), cyanidin-3-glucoside (5%) and cyanidin-3-arabinoside (11%)) was previously reported by Foley and Debnath (2007). In this study investigated lingonberries had similar anthocyanins profile to what was previously reported by Kähkönen et al. (2003), Lee with Finn (2012) and Isaak, et al. (2017) without the additional minor peaks reported by Ek et al. (2006) and Lätti et al. (2011).

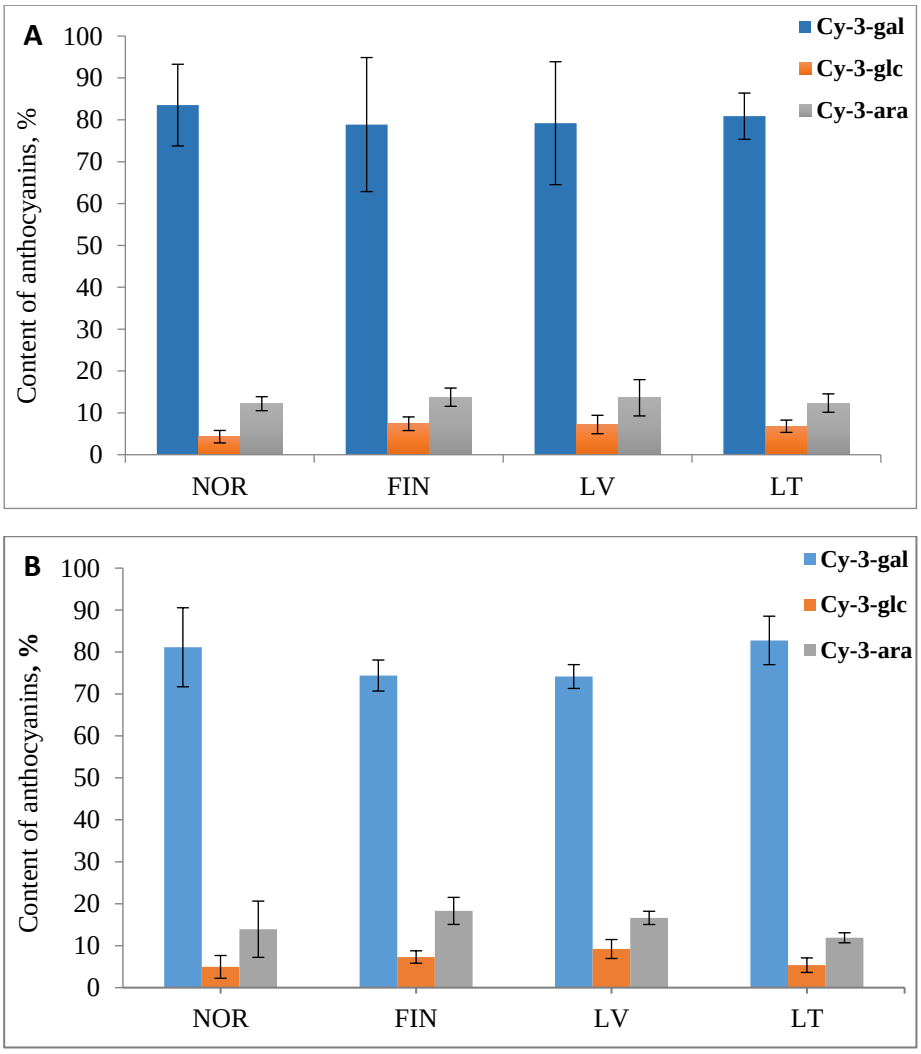


**Figure 4.** The mean content of anthocyanins in lingonberry samples (%) harvested in 2019 (A) and 2020 (B). Different letters above the same color bars indicate significant differences between the mean values ($p < 0.05$).

Lingonberry samples from all four countries (NOR, FIN, LTV and LTU) contained similar proportions of identified anthocyanins (cyanidin-3-galactoside, cyanidin-3-glucoside and cyanidin-3-arabinoside).

*Antioxidant activity (AA)*

*Bilberries.* The antioxidant activity of berry samples was evaluated using FRAP and ABTS assays. In ABTS assay antioxidants suppress the generation of a blue-green ABTS radical cation by electron donation radical scavenging, whereas in the FRAP assay there are no free radicals involved, but the reduction of ferric-to-ferrous iron is monitored.

In 2019 the FRAP of bilberry samples ranged from 36.0 (LTU B10) to 57.7 µmol TE/g fw (NOR B2) and in 2020 from 35.1 (LTU B12) to 49.1 µmol TE/g fw (NOR B2) (Figure 5). Both years of investigation, the highest mean FRAP value had berry samples collected in Norway (50.6 µmol TE/g and 46.6 µmol TE/g fw in 2019 and 2020, respectively), followed by samples collected in Latvia (45.0 µmol TE/g and 46.3 µmol TE/g fw in 2019 and 2020, respectively). Bilberry samples from Lithuania had the lowest mean FRAP values (41.2 µmol TE/g and 40.2 µmol TE/g fw in 2019 and 2020, respectively). FRAP results obtained in our study are close to previously reported (53 and 57 µmol TE/g fw) in *V. myrtillus* fruits (Nestby et al., 2011).

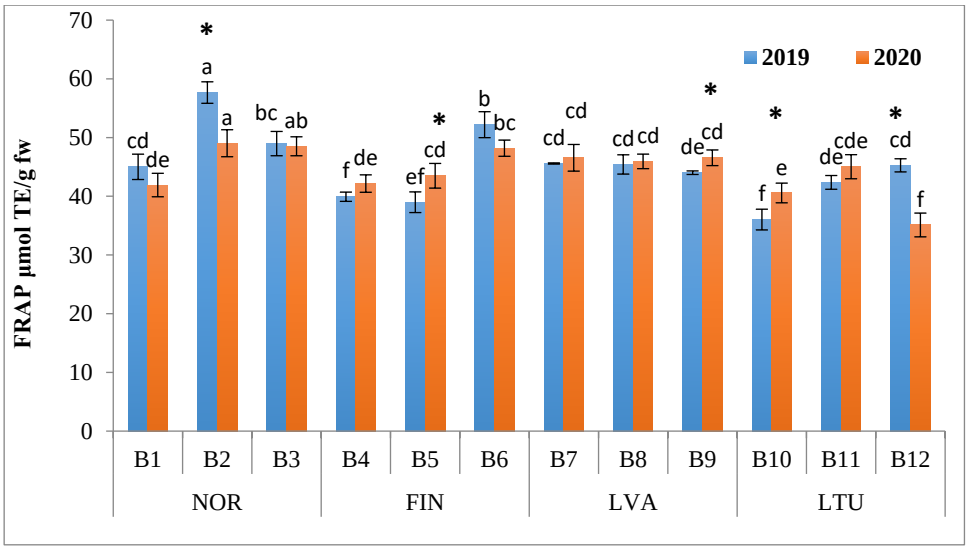


**Figure 5.** Ferric reducing antioxidant power (FRAP) of bilberries (µmol TE/g fw). Different letters above the same color bars indicate significant differences between the mean values ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).

Berries showed higher antioxidant activity in ABTS reaction system (Figs. 6 and 8). ABTS RSA of bilberries ranged from 60.9 (LTU B12 in 2020) to 106.0 µmol TE/g fw (NOR B2 in 2020). In 2019, the highest mean ABTS RSA had berry samples collected in Norway (95.1 µmol TE/g fw), followed by samples collected in Finland (81.3 µmol TE/g fw), whereas in 2020, the highest mean ABTS RSA had berry samples from Latvia (89.9 µmol TE/g fw) followed by samples from Norway (83.3 µmol TE/g fw) (Fig. 6).

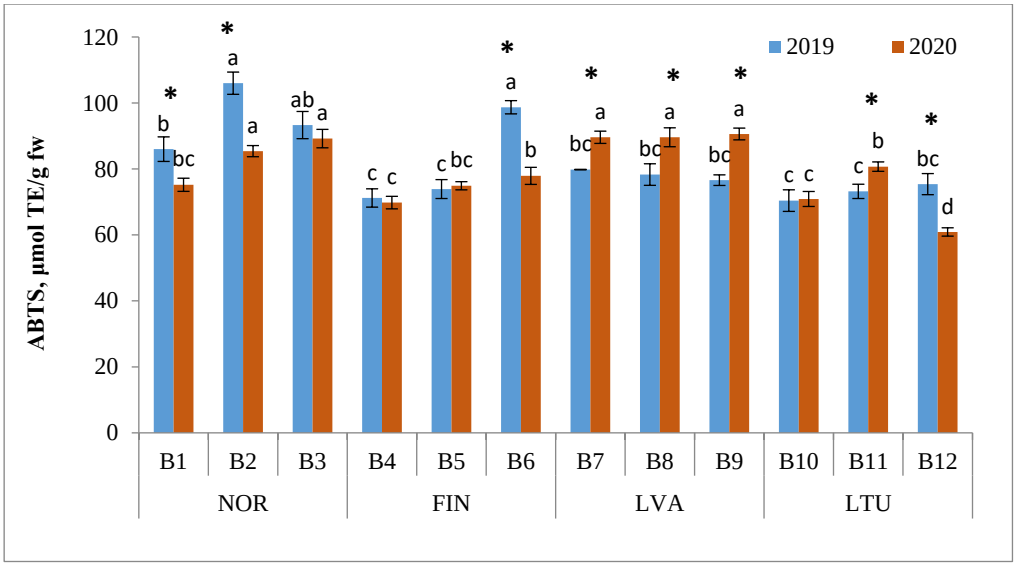


**Figure 6.** ABTS radical scavenging activity (RSA) of bilberries (µmol TE/g fw). Different letters above the same color bars indicate significant differences between the mean values ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).

In agreement with previous findings (Giovanelli, Buratti, 2009; Dincheva, Badjakov, 2016), a high positive correlation was found between TPC and antioxidant activity of the bilberry samples (R=0.88 and 0.91 as determined by the FRAP and ABTS assays, respectively), whereas the correlation between TAC and antioxidant activity was somewhat lower (R=0.65 and 0.60 as determined by the FRAP and ABTS assays, respectively).

Previously, Giovanelli and Buratti (2009) investigated cultivated blueberries (*Vaccinium corymbosum*) and wild bilberries (*Vaccinium myrtillus*) and reported that the antioxidant capacity of berries strongly correlated with the content of total anthocyanins and total phenolics. However, contrary to our findings, higher correlation coefficient was found between the antioxidant capacity and total anthocyanins content (R=0.93) than between the antioxidant capacity and total phenolic content (R=0.89) of berries (Giovanelli, Buratti, 2009). Uleberg et al. (2012) also reported quite strong correlations between anthocyanins, total phenolics and antioxidant activity of bilberries.

*Lingonberries*. It was reported previously that lingonberry occupies a significant position in the antioxidant and antimicrobial capacity ranking of *Vaccinium*-derived species (Grace et al., 2014). However, in our study the mean FRAP and ABTS RSA values of lingonberry samples were approximately 22% and 26% lower than respective values measured for bilberries. In 2019, the FRAP values of lingonberries ranged from 32.6 (NOR L1) to 43.8 µmol TE/g fw (NOR L3) and in 2020, values were lower and ranged from 27.1 (NOR L2) to 40.8 µmol TE/g fw (NOR L1) (Figure 7). In 2019, the highest mean FRAP value had berry samples collected in FIN (41.4 µmol TE/g fw), followed by samples from NOR (38.8 µmol TE/g fw) and in 2020, the highest values had berries from LTU and NOR (34.3 and 34.0 µmol TE/g fw, respectively).

ABTS RSA of lingonberries varied from 35.3 µmol TE/g fw (FIN L4 in 2020) to 88.8 µmol TE/g fw (NOR L3 in 2019) (Figure 8). In 2019, the highest ABTS RSA values were measured in berries from NOR, followed by berries from FIN, whereas in 2020 the highest values had berries from NOR and LTU.

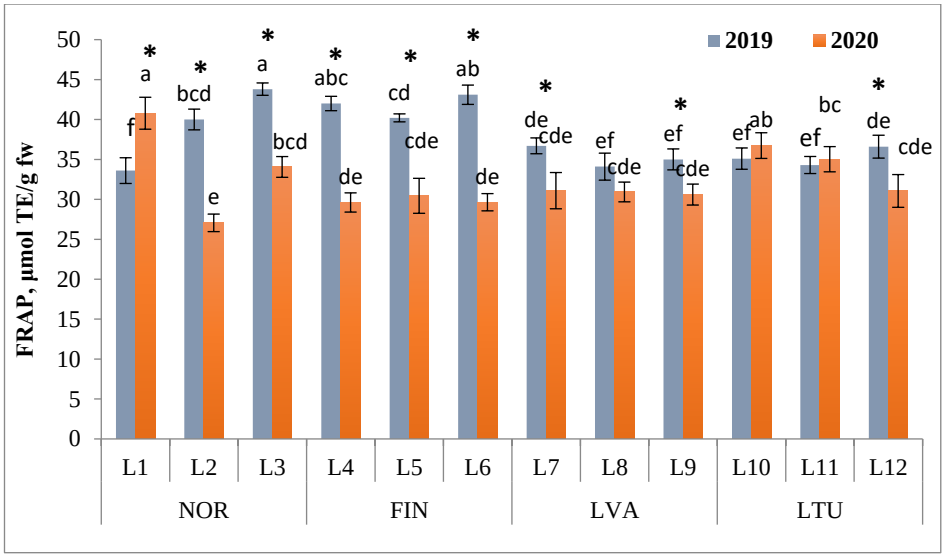


**Figure 7.** Ferric reducing antioxidant power (FRAP) of lingonberries (µmol TE/g fw). Different letters above the same color bars indicate significant differences between the mean values ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).

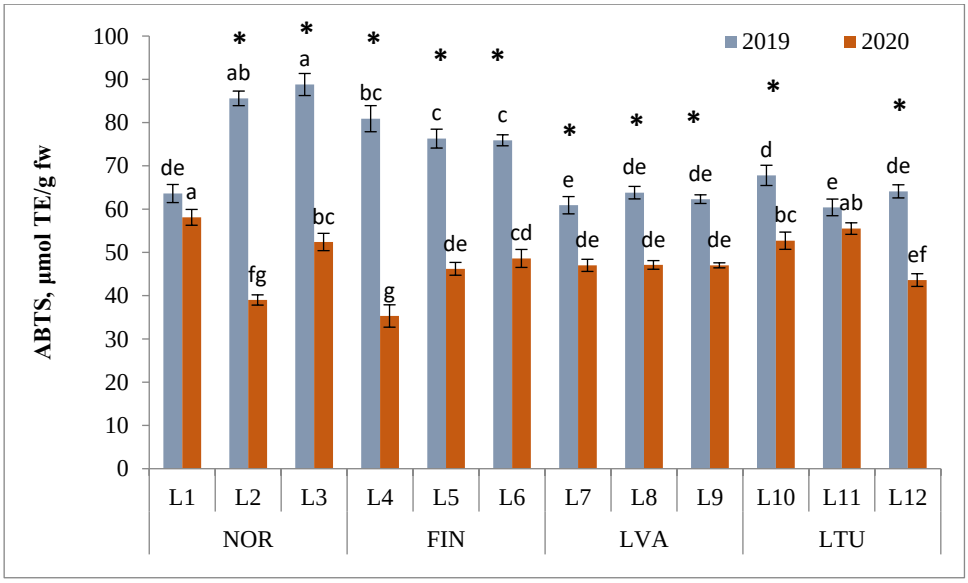


**Figure 8.** ABTS radical scavenging activity (RSA) of lingonberries (µmol TE/g fw). Different letters above the same color bars indicate significant differences between the mean values ($p < 0.05$). Significant differences between 2019 and 2020 are indicated by asterisks (*) ($p < 0.05$).

Similar as observed in bilberries, a high positive correlation was also found between TPC and antioxidant activity of investigated lingonberries (R=0.93 and 0.92 as determined by the FRAP and ABTS assays, respectively). In the present study no correlation was found between TAC and antioxidant activity of lingonberries, which suggest that phenolic compounds other than anthocyanins are responsible for the majority of the antioxidant activity of these berries. Our findings agree with those previously published by Nestby et al. (2011) who reported that there was no correlation between antioxidant activities and anthocyanin content among 18 lingonberry genotypes (R =-0.141). On the other hand, it was shown previously that each of the three anthocyanins found in lingonberries (cyanidin-3-galactoside, cyandin-3-glucoside, and cyanidin-3-arabinoside) protected cardiac cells from

oxidative stress-induced apoptosis and may have cardioprotective effects as a dietary modification (Isaak et al., 2017).

## 2.3 Summary

The pH values of investigated bilberries and lingonberries varied from 2.94 to 3.47 and from 2.66 to 3.03, respectively. SS content of bilberries and lingonberries varied within similar range - from 9.4 to 15.8 Brix° and from 10.4 to 15.3 Brix°, respectively. In 2020, the pH values and SS content of berries were significantly lower than in 2019, showing that weather conditions have high influence on these parameters.

The TPC of bilberries ranged from 452 to 902 mg/100 g fw. The mean TPC of bilberries from the Norway (the most northern country covered in the study) was the highest, whereas the mean TPC of samples from the most southern country (Lithuania) was the lowest. The TPC of lingonberries was within similar range as in bilberries and varied from 477 to 776 mg/100 g fw. In 2020, the average TPC values of lingonberry samples were up to 25% lower.

The TAC values of investigated bilberry samples varied from 232.7 to 475.5 mg/100 g fw and were somewhat lower in 2020 than in 2019.

The TAC in lingonberries was significantly lower (19.8 - 57.0 mg/100 g fw) than in bilberries. In this study lingonberry samples from NOR and FIN, both years of investigation, had the highest TAC (varied from 41.7 to 57.0 mg/100 g fw).

There were significant yearly variations in the TPC and TAC values of investigated berries, suggesting that although genotype affects the TPC and TAC in berries, their final content also depends on weather conditions.

Using HPLC-DAD, 15 anthocyanins were identified in bilberries and 3 in lingonberries. In bilberries from NOR and FIN the major anthocyanin was Dp-3-ara, followed by Dp-3-gal and Dp-3-glc whereas in bilberries from LTV and LTU - Cy-3-glc, followed by Cy-3-gal and Dp-3-glc. Both years of investigation, bilberries from LTV and LTU had significantly lower contents of Dp-3-gal and Dp-3-glc than berries from NOR and FIN, but higher contents of Pn-3-glc.

Cy-3-gal, comprising up to 83.5% of the total anthocyanins present, was the dominant anthocyanin in lingonberries followed by Cy-3-ara and Cy-3-glc.

A high positive correlation was found between TPC and antioxidant activity of the bilberry samples (R=0.88 and 0.91 as determined by the FRAP and ABTS assays, respectively), whereas the correlation between TAC and antioxidant activity was lower (R=0.65 and 0.60 as determined by the FRAP and ABTS assays, respectively). Similar as observed in bilberries, a high positive correlation was found between TPC and antioxidant activity of lingonberries (R=0.93 and 0.92 as determined by the FRAP and ABTS assays, respectively). However, no correlation was found between TAC and antioxidant activity of lingonberries.

# 3. Quality results of berries obtained by Centria

Centria obtained bilberry and lingonberry samples from four different countries: Norway, Finland, Lithuania and Latvia in 2020. The berries were freeze-dried and their total polyphenol content, anthocyanin content, proanthocyanidin content, flavonoid content and antioxidant capacity measured. Due to mechanical failure of HPLC, individual anthocyanidins could not be analyzed from berries. The purpose of the study was to see, if there are differences in the quality of berries from different regions.

## 3.1 Materials and Methods

Folin-Ciocalteu's phenol reagent, sodium carbonate, aluminum chloride, potassium acetate, 4-(dimethylamino)cinnamaldehyde (DMAC), copper(II) chloride dihydrate, ammonium acetate, neocuproine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, gallic acid (synthesis grade), quercetin (secondary standard), epicatechin (primary reference standard) and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (97%), were purchased from Merck. Sodium acetate was purchased from Honeywell and kuromanin chloride (cyanidin-3-O-glucoside chloride) (≥96%) from Extrasynthese. Potassium chloride, trifluoroacetic acid and acetonitrile were purchased from VWR Chemicals and methanol from Fisher Scientific. Finally, ethanol Etax A was purchased from Altia Oyj.

### 3.1.1 Samples

Bilberry and lingonberry samples were obtained from four different countries: Norway (Tromsø region), Finland (Oulu region), Lithuania and Latvia. The berries were collected in summer/fall 2020 at their ripest stage. Three sample batches were obtained from every country except just one from Latvia. The berry batches were collected at least 10 km apart. The collected berries were frozen and delivered to Centria through University of Oulu in a package filled with dry ice. Once received, the berries were moved into a freezer.

### 3.1.2 Pretreatment and extraction

The berry samples were dried using a Labogene Coolsafe Touch 100-9 freeze dryer in vacuum (<0.5 hPa) and cooling the shelves to -10 °C. Due to big drying batches, the drying took 1.5 – 2 weeks and the freeze-drier had to be stopped and melted during the process.

After freeze drying, the berries were grinded using IKA A11 analytical mill and stored in plastic jars at RT. The remaining moisture content was determined by drying 1.5 g of berry powder at 105±5 °C over night (at least 15h). A duplicate determination was made from each sample.

The berries were extracted using an ultrasonic-assisted extraction method studied and optimized in the beginning of the project. One (1) g of freeze-dried berry powder was weight into an Erlenmeyer flask with a screw cap and 25 ml of 75% aqueous ethanol containing 0.5% of TFA was added. The extraction was carried out with ultrasonic bath at RT for 30 min. However, due to the effect of ultrasound, the temperature increased slightly above 30 °C.

After the extraction, the extract was moved into a centrifuge tube and centrifuged at 4000 rpm for 10 minutes. Five (5) ml of the supernatant was recovered and the rest of the supernatant was moved into the waste. The residue of the berry powder was transferred back to the Erlenmeyer flask and extracted

again with 25 ml of 75% aqueous ethanol containing 0.5% of TFA. The extract was centrifuged and 5 ml of supernatant recovered. Finally, the residue was extracted for the third time with 25 ml of 75% aqueous ethanol containing 0.5% of TFA. The extract was centrifuged and 5 ml of supernatant recovered. The supernatants (5 ml) from three extractions were combined. A duplicate extraction was made from each berry sample.

### 3.1.3 Analysis

Total polyphenol content, anthocyanin content, flavonoid content, proanthocyanidin content and CUPRAC, DPPH and ABTS antioxidant capacities were measured from bilberries and lingonberries (Tab. 1).

**Table 1**. Summary of analysis methods.

| Analysis method | Assay | Unit |
|---|---|---|
| **Polyphenol content (TPC)** | Folin-Ciocalteu | mg GAE/g dw |
| **Total anthocyanin content (TAC)** | pH differential | mg $C_3G$/g dw |
| **Total flavonoid content (TFC)** | Al-Cl | mg QE/g dw |
| **Total proanthocyanidin content (TPC)** | DMAC | mg ECE/g dw |
| **Antioxidant capacity (AC)** | CUPRAC, DPPH, ABTS | mmol TE/g dw |

*Total polyphenol content*

Total polyphenol content was determined with Folin-Ciocalteu's method. Half (0.5) ml of extract was pipetted into an Eppendorf-tube. Then, 2.5 ml of 10% Folin-Ciocalteu-solution and 2.0 ml of 75 g/l sodium carbonate solution was added. The tube was mixed and placed into a 50 °C water bath for 5 min. Then, the tube was quickly cooled near RT before the measurements. The absorbance was measured with Shimadzu UV-1800 spectrophotometer at 760 nm against reagent blank. A duplicate determination was made from each extract. Gallic acid (0.01 – 0.10 mg/ml) was used as a standard and the results are expressed as gallic acid equivalents per dry weight (mg GAE/g dw).

*Total anthocyanin content*

Total anthocyanin content was measured by pH differential anthocyanin method. One (1.0) ml of extract was pipetted into two Eppendorf tubes. Four (4) ml of 0.4 M sodium acetate buffer solution (pH 4.5) was added to the tube and 4 ml of 0.025 M potassium chloride buffer solution (pH 1) to the other tube. The tubes were mixed and left to react 20 min in the dark. The absorbance was measured with Shimadzu UV-1800 spectrophotometer at 700 nm against ultrapure water. A duplicate determination was made from each extract. The results were calculated as cyanidin-3-O-glucoside equivalents using molar absorptivity of 26 900 l/mol·cm and molecular mass of 449.2 g/mol. The results are expressed as cyanidin-3-*O*-glucoside equivalents per dry weight (mg $C_3G$/g dw).

*Total flavonoid content*

Total flavonoid content was determined with aluminum chloride assay. Half (0.5) ml of extract was pipetted into an Eppendorf tube. One and half (1.5) ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of ultrapure water were added to the tube and mixed. The tube was left to react 30 min in the dark. The absorbance was measured with Shimadzu UV-1800 spectrophotometer at 415 nm against reagent blank. A duplicate determination was made from each extract. Quercetin (0.01 – 0.15 mg/ml) was used as a standard and the results are expressed as quercetin equivalents per dry weight (mg QE/g dw).

*Total proanthocyanidin content*

Total proanthocyanidin content was determined with DMAC assay. One (1) ml of extract was pipetted into an Eppendorf tube and 3 ml of 1 g/l DMAC reagent was added. The tube was mixed and left to react 30 min in the dark. The absorbance was measured with Shimadzu UV-1800 spectrophotometer at 640 nm against reagent blank. A duplicate determination was made from each extract. Epicatechin (0.002 – 0.016 mg/ml) was used as a standard and the results are expressed as epicatechin equivalents per dry weight (mg ECE/g dw).

*Antioxidant capacity*

Antioxidant capacity was determined by three methods: CUPRAC, DPPH and ABTS. All of them produce colorful solutions but are based on different redox reagents. Trolox was used as a calibration standard and the results are expressed as Trolox equivalents per dry weight (mmol TE/g dw).

In CUPRAC assay, 1 ml of 0.01 M $CuCl_2$, 1 ml of 1.0 M $NH_4$(Ac) (pH 7), 1 ml of 0.0075 M neocuproine and 1 ml of ultrapure water were added into an Eppendorf tube. Finally, 0.1 ml of extract was added and the tube was closed and mixed. The tube was left to react for 30 min in the dark. The absorbance was measured with Shimadzu UV-1800 spectrophotometer at 450 nm against reagent blank. A duplicate determination was made from each extract. Trolox (0.01 – 0.25 mM) was used as a standard.

In DPPH assay, 2 ml of 0.06 mM DPPH -solution (absorption adjusted to 0.800±0.010) was added into an Eppendorf tube and mixed with 20 µl of extract. The tube was left to react for 30 min in the dark. The absorbance was measured with Shimadzu UV-1800 spectrophotometer at 515 nm against methanol. A duplicate determination was made from each extract. Trolox (0.2 – 3.0 mM) was used as a standard.

In ABTS assay, 2.85 ml of working solution [40 ml ethanol mixed with 1 ml of 1:1 $ABTS^{\bullet+}$(7.0 mM): $K_2S_2O_8$(2.45 mM)] was added into an Eppendorf tube and mixed with 0.15 ml of extract. The tube was left to react for 30 min in the dark. The absorbance was measured with Shimadzu UV-1800 spectrophotometer at 734 nm against ethanol. A duplicate determination was made from each extract. Trolox (0.05 – 0.40 mM) was used as a standard.

## 3.2 Results and discussion

*Moisture content*. The moisture content of freeze-dried bilberries varied between 11 – 16 m-%. With freeze-dried lingonberries, the moisture content varied between 10 – 24 m-%. The moisture content of lingonberries was in some samples higher than aimed for and it could cause early molding and microbe growth. To prevent this, the samples were extracted within a week from drying and analyzed within a week from extraction. The results were calculated per dry weight in order to eliminate the effect of the varying moisture content.

During the freeze drying, it was observed that lingonberries took more time to dry than bilberries. This was probably caused by a thicker berry peel of lingonberries, which inhibits the evaporation of water. It was also noticed that depending on the shelf (upper and lower shelf of freeze dryer), the drying occurred at different rates. Therefore, the locations of the samples were switched between the shelves when the dryness of the berries were tested.

*Bilberries.* The results of bilberries are shown in Table 2 and 4. Total polyphenol content of bilberries picked from Finland varied between 52 – 66 mg GAE/g dw, from Norway 64 – 71 mg GAE/g dw, from Lithuania 43 – 69 mg GAE/g dw and from Latvia polyphenol content of 61 mg GAE/g dw was obtained. Thus, slightly higher total polyphenol contents were obtained in bilberries picked in Norway. The

obtained polyphenol contents were higher than 34.7 – 41.9 mg GAE/g dm reported by Bujor et al. (2016) and 3993 mg/100g dm reported by Skoczeń-Słupska et al. (2016).

Total anthocyanin content of bilberries picked from Finland varied between 36 – 47 mg GAE/g dw, from Norway 32 – 36 mg GAE/g dw, from Lithuania 26 – 53 mg GAE/g dw and from Latvia anthocyanin content of 43 mg GAE/g dw was obtained. Thus, similar trend could not be observed with total anthocyanins as with polyphenols. The contents were higher than 1649 mg/100g dm reported by Skoczeń-Słupska et al. (2016) and 2878 mg/100g dw reported by Lätti et al. (2008).

Fairly low total flavonoid content (under 9 mg QE/g dw) and total proanthocyanidin content (under 6 mg ECE/ g dw) were measured from bilberries The flavonoids are a major polyphenol group in berries and anthocyanins are part of that group together with flavanones, isoflavones, flavones, flavonols and flavanols (Ignat et al., 2011). Thus, total flavonoid content is erroneously low as the content should be higher than the anthocyanin content. Based on the literature, aluminum chloride assay is effective only to flavones and flavanones, so the result obtained doesn't actually represent total flavonoid content (Chang et al., 2002).

**Table 2.** Total contents measured from bilberries from different regions.

| Sample | Anthocyanin content (mg $C_3G$/g dw) | Polyphenol content (mg GAE/g dw) | Flavonoid content (mg QE/g dw) | Proanthocyanidin content (mg ECE/g dw) |
|---|---|---|---|---|
| **Finland 1** | 36,67 | 55,02 | 7,10 | 2,61 |
| **Finland 2** | 35,51 | 52,03 | 6,51 | 2,81 |
| **Finland 3** | 46,63 | 65,58 | 7,80 | 2,97 |
| **Norway 1** | 35,18 | 71,34 | 7,78 | 5,02 |
| **Norway 2** | 31,53 | 63,94 | 6,96 | 4,91 |
| **Norway 3** | 35,76 | 70,08 | 8,19 | 5,09 |
| **Lithuania 1** | 39,35 | 54,01 | 6,79 | 1,70 |
| **Lithuania 2** | 53,08 | 69,32 | 8,65 | 2,72 |
| **Lithuania 3** | 25,79 | 42,73 | 5,30 | 1,64 |
| **Latvia 1** | 42,54 | 60,71 | 7,63 | 2,49 |

**Standard deviation in anthocyanin content was less than 4%, in polyphenol and flavonoid content less than 5% and in proanthocyanidin content less than 7%.*

Total polyphenol content and total anthocyanin content do not correlate fully with each other. The highest polyphenol contents in bilberries were measured in the following samples: Norway 1, Norway 3 and Lithuania 2. The highest anthocyanin contents were detected in the following samples: Lithuania 2, Finland 3 and Latvia 1. However, some correlation can be seen as both the lowest polyphenol and anthocyanin content were measured in sample 'Lithuania 3'.

*Lingonberries.* The results of lingonberries are shown in Table 3 and 5. Total polyphenol content of lingonberries picked from Finland varied between 47 – 52 mg GAE/g dw, from Norway 53 – 78 mg GAE/g dw, from Lithuania 51 – 56 mg GAE/g dw and from Latvia polyphenol content of 52 mg GAE/g dw was obtained. The polyphenol content of lingonberries was in the same level than with bilberries, and the highest polyphenol contents were observed in Norwegian lingonberries. The measured polyphenol contents were higher than 15.4 – 17.2 mg GAE/g dm reported by Bujor et al. (2018).

Fairly low total anthocyanin content (under 7 mg $C_3G$/g dw) and flavonoid content (under 7 mg QE/g dw) were measured in lingonberries. With lingonberries, the flavonoid content was typically greater than the anthocyanin content as lingonberries contain only three anthocyanins (Lee, Finn, 2012).

Slightly higher amounts (over 8 mg ECE/g dw) of proanthocyanidins were detected in lingonberries than in bilberries.

**Table 3.** Total contents measured from lingonberries from different regions.

| Sample | Anthocyanin content (mg $C_3G$/g dw) | Polyphenol content (mg GAE/g dw) | Flavonoid content (mg QE/g dw) | Proanthocyanidin content (mg ECE/g dw) |
|---|---|---|---|---|
| **Finland 1** | 6,02 | 46,73 | 3,92 | 8,61 |
| **Finland 2** | 4,72 | 49,81 | 3,78 | 9,29 |
| **Finland 3** | 4,02 | 51,51 | 3,91 | 9,59 |
| **Norway 1** | 5,38 | 78,33 | 6,81 | 14,87 |
| **Norway 2** | 5,65 | 52,62 | 4,64 | 9,56 |
| **Norway 3** | 6,33 | 57,78 | 4,12 | 11,28 |
| **Lithuania 1** | 1,96 | 51,08 | 4,19 | 9,15 |
| **Lithuania 2** | 3,93 | 52,30 | 3,41 | 9,40 |
| **Lithuania 3** | 1,96 | 55,59 | 4,20 | 10,99 |
| **Latvia 1** | 2,82 | 51,85 | 3,66 | 9,36 |

**Standard deviation in anthocyanin content was less than 11%, in flavonoid content less than 6% and in polyphenol and proanthocyanidin content less than 2%.*

*Antioxidant capacity.* Antioxidant capacity was at the same level with bilberries and lingonberries. The antioxidant capacity of bilberries varied between 0.455 – 0.755 mmol TE/g dw, 0.306 – 0.413 mmol TE/g dw and 0.191 – 0.276 mmol TE/g dw with CUPRAC, DPPH and ABTS, consecutively. The antioxidant capacity of lingonberries varied between 0.514 – 0.841 mmol TE/g dw, 0.325 – 0.468 mmol TE/g dw and 0.204 – 0.308 mmol TE/g dw with CUPRAC, DPPH and ABTS, consecutively. The highest antioxidant capacities were measured in Norwegian berries. Comparison of results to the literature is difficult, because in many publications the results have been expressed per fresh weight, which can vary depending on the moisture content.

**Table 4.** Antioxidant capacities measured from bilberries from different regions.

| Sample | CUPRAC antioxidant capacity (mmol TE/g dw) | DPPH antioxidant capacity (mmol TE/g dw) | ABTS antioxidant capacity (mmol TE/g dw) |
|---|---|---|---|
| **Finland 1** | 0,592 | 0,358 | 0,236 |
| **Finland 2** | 0,560 | 0,349 | 0,227 |
| **Finland 3** | 0,708 | 0,407 | 0,265 |
| **Norway 1** | 0,755 | 0,413 | 0,269 |
| **Norway 2** | 0,683 | 0,401 | 0,258 |
| **Norway 3** | 0,754 | 0,407 | 0,268 |
| **Lithuania 1** | 0,573 | 0,346 | 0,231 |
| **Lithuania 2** | 0,754 | 0,413 | 0,276 |
| **Lithuania 3** | 0,455 | 0,306 | 0,191 |
| **Latvia 1** | 0,641 | 0,373 | 0,252 |

**Standard deviation in CUPRAC, DPPH and ABTS antioxidant capacity was less than 4%.*

All three assays (CUPRAC, DPPH and ABTS) correlated quite well with each other and total polyphenol content. In both bilberries and lingonberries, the highest antioxidant capacities were obtained using CUPRAC assay followed by DPPH and ABTS. However, depending on the species studied and the

compounds present in it, the response of the antioxidant assay varies (Dudonné et al., 2009; Apak et al.,2007). Therefore, it is material and compound specific, which assay gives the highest result.

**Table 5.** Antioxidant capacities measured from lingonberries from different regions.

| Sample | CUPRAC antioxidant capacity (mmol TE/g dw) | DPPH antioxidant capacity (mmol TE/g dw) | ABTS antioxidant capacity (mmol TE/g dw) |
|---|---|---|---|
| **Finland 1** | 0,514 | 0,325 | 0,204 |
| **Finland 2** | 0,543 | 0,329 | 0,214 |
| **Finland 3** | 0,572 | 0,339 | 0,224 |
| **Norway 1** | 0,841 | 0,468 | 0,308 |
| **Norway 2** | 0,564 | 0,335 | 0,224 |
| **Norway 3** | 0,619 | 0,352 | 0,237 |
| **Lithuania 1** | 0,548 | 0,364 | 0,219 |
| **Lithuania 2** | 0,552 | 0,367 | 0,225 |
| **Lithuania 3** | 0,598 | 0,394 | 0,240 |
| **Latvia 1** | 0,555 | 0,395 | 0,231 |

**Standard deviation in CUPRAC and ABTS antioxidant capacity was less than 2% and in DPPH antioxidant capacity less than 5%.*

*Differences between countries.* It is assumed that the long light period and northern location improves the production of plant phytochemicals (Ștefănescu et al., 2020). Same was also observed in this study. The highest polyphenol, flavonoid and proanthocyanidin contents were measured in Norwegian berries. However, Norwegian bilberries had the lowest anthocyanin content compared to bilberries from other countries, even though Norwegian lingonberries had the highest anthocyanin content. The unexpectedly low anthocyanin content of Norwegian bilberries could result from sampling, weather conditions or unripen berries.

The figures 1 - 4 show the average contents of bilberries and lingonberries picked from different regions allowing better detection of differences between countries. The error bars include the intra-country variations.

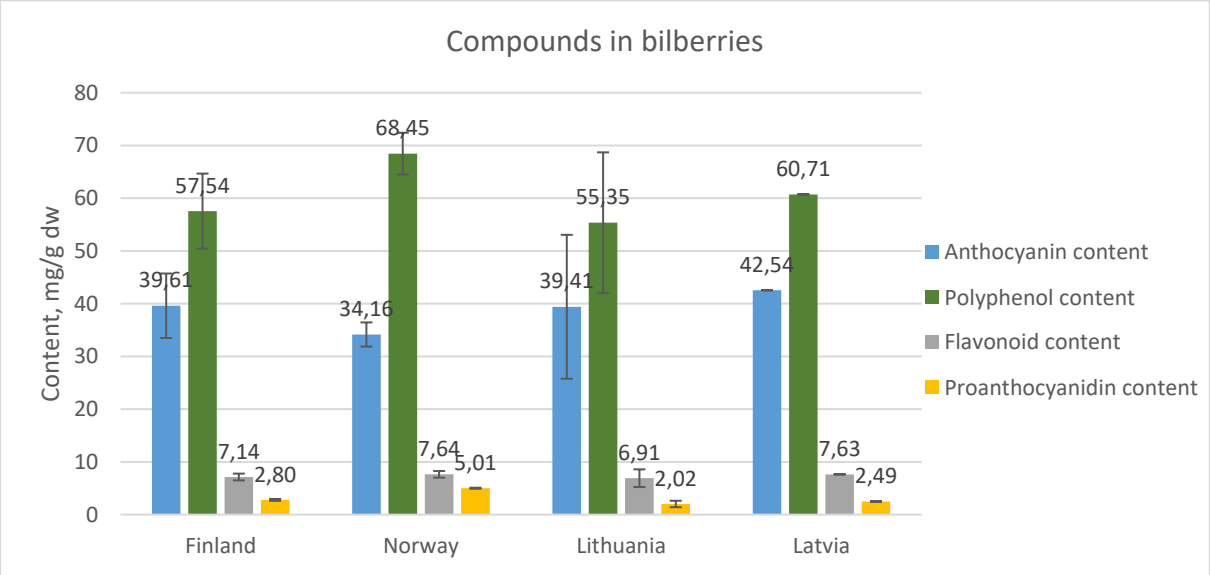


**Figure 1.** Average total anthocyanin, polyphenol, flavonoid and proanthocyanidin content of bilberries from different countries.

As seen in the figure 1, Norwegian bilberries have the highest average polyphenol content, flavonoid content and proanthocyanidin content but the lowest anthocyanin content. With other countries, there aren't any notable differences. Same trend can be observed in figure 2 regarding antioxidant capacity. Norwegian bilberries have the highest average antioxidant capacity with all three methods, but between Finland, Lithuania and Latvia there aren't notable differences.

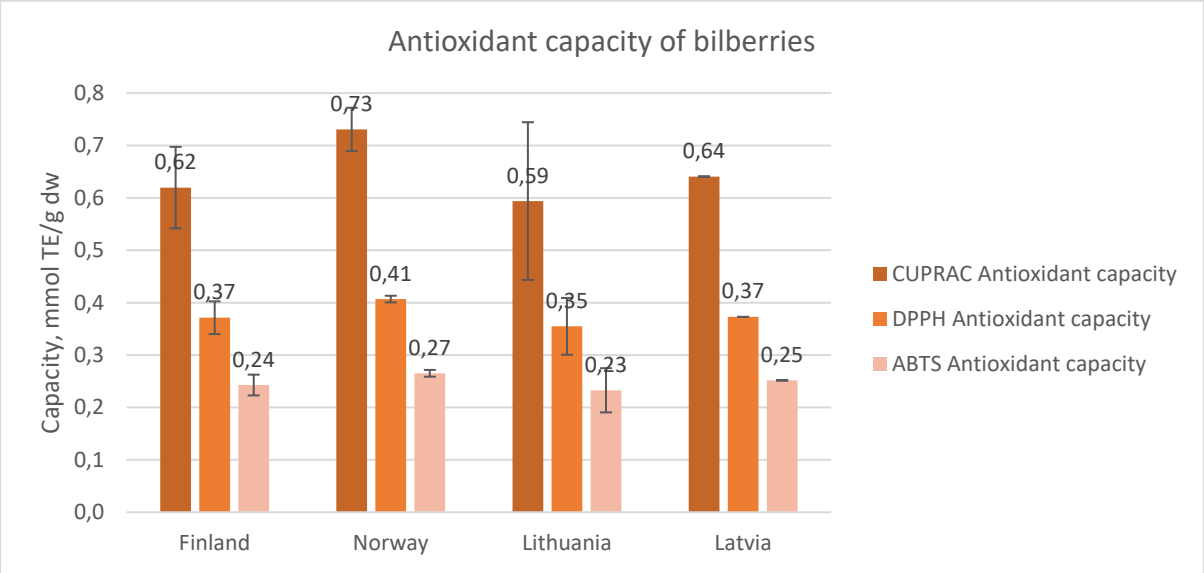


**Figure 2.** Average CUPRAC, DPPH and ABTS antioxidant capacity of bilberries from different countries.

Similar trend continues with lingonberries that described with bilberries. Figure 3 demonstrates how Norwegian lingonberries have the highest average polyphenol content, flavonoid content and proanthocyanidin content and in this case, also the highest anthocyanin content. Between other countries, there aren't any notable differences. However, Norwegian lingonberries doesn't have the highest average antioxidant capacity with all three methods, as Lithuanian and Latvian lingonberries also have quite high DPPH antioxidant capacity (Fig. 4).

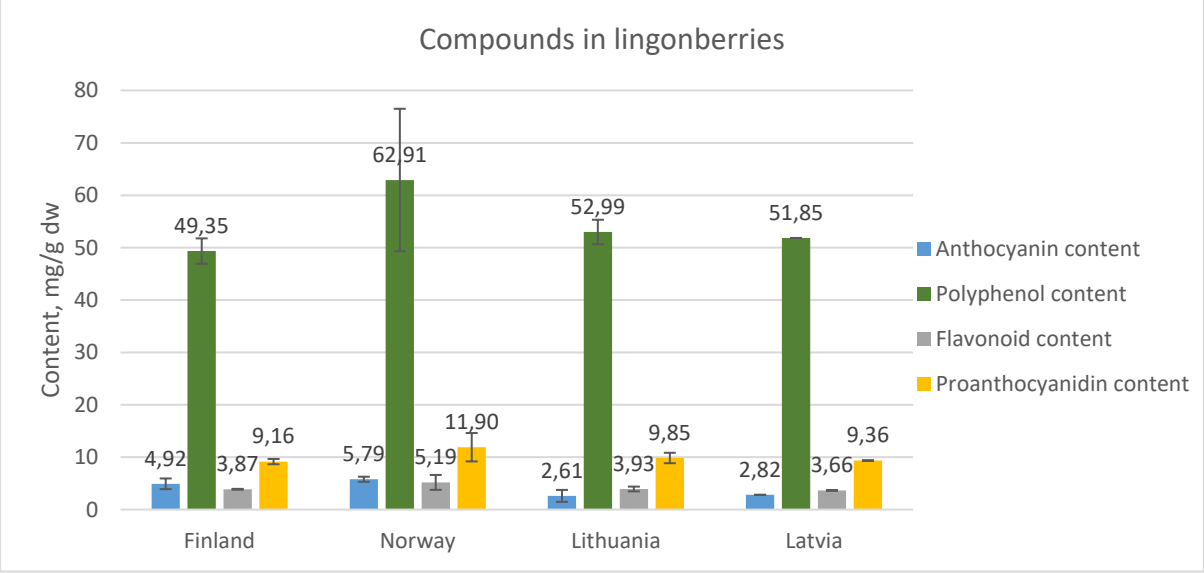


**Figure 3.** Average total anthocyanin, polyphenol, flavonoid and proanthocyanidin content of lingonberries from different countries.

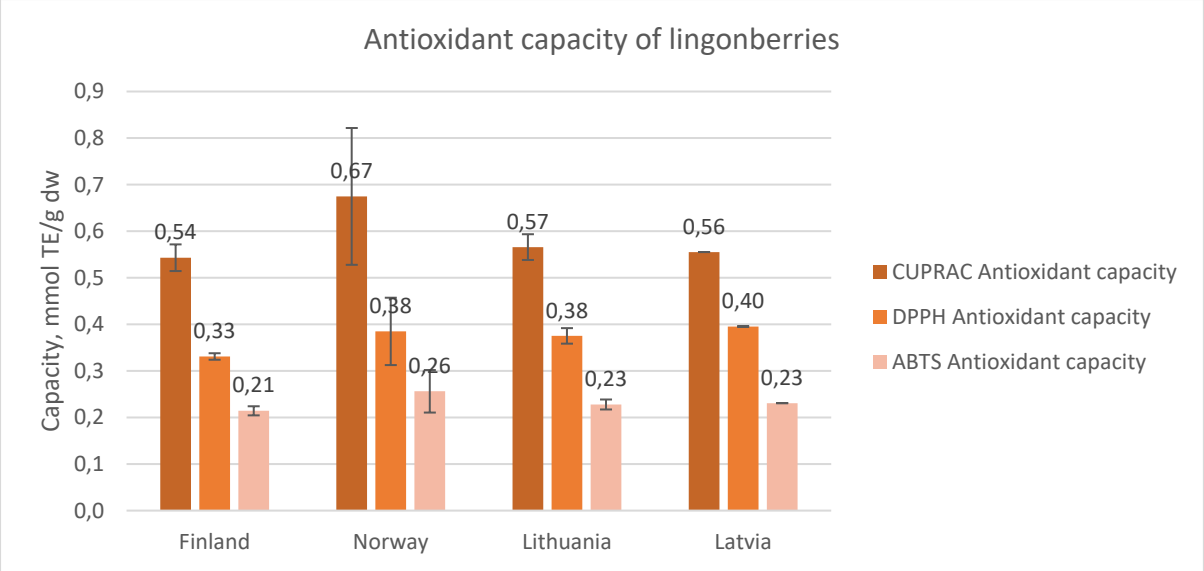


**Figure 4.** Average CUPRAC, DPPH and ABTS antioxidant capacity of lingonberries from different countries.

There are notable variations in the quality of berries inside the countries. Lithuanian bilberries and Norwegian lingonberries have large intra-country variations in antioxidant capacities and in polyphenol, anthocyanin, proanthocyanidin and flavonoid contents. This may be due to significant intra-country differences in growing conditions (weather and soil) or surrounding environments. The berries might have also been picked at different stage of ripeness.

## 3.3 Summary

Between Finland, Lithuania and Latvia there weren't notable differences in quality of lingonberries or bilberries. Similar results were obtained in polyphenol, anthocyanin, flavonoid and proanthocyanidin content and in antioxidant capacity. Norwegian berries had the highest polyphenol, flavonoid and proanthocyanidin content and antioxidant capacity. Exceptionally, Norwegian bilberries had the lowest anthocyanin content compared to other countries, even though Norwegian lingonberries had the highest.

It seems that in northern latitudes, polyphenol content, flavonoid content and proanthocyanidin content are higher. However, this phenomenon could not be confirmed with anthocyanins. More sample data from different years would be needed to confirm, if the contents increase the northern the berries are grown, because many other factors besides geographical location affects the chemical contents. Also, the differences in quality were not large between the countries and intra-country variations were notable.

UV-Vis-based quality characterization methods are fast and easy to use, and therefore highly suitable for this type of comparisons. However, the methods are not as precise as liquid or gas chromatography-based methods. Thus, the made observations are shallow and complementary data from other analysis methods would be needed to give wider perspective.

# 4. Conclusions

In both LAMMC's and Centria's reports, bilberries and lingonberries were found to contain equal amounts of polyphenols and that bilberries contained significantly more anthocyanins than lingonberries. Norwegian bilberries were found to have the highest polyphenol content. Centria also found the polyphenol content of Norwegian lingonberries to be the highest, but LAMMC reported polyphenol content of Norwegian lingonberries to be similar with other countries.

Centria and LAMMC both reported the highest anthocyanin content of lingonberries in Norwegian and Finnish berries. However, Centria found the anthocyanin content of bilberries to be the lowest in Norwegian berries. LAMMC noted that in addition to the genotype of berries, polyphenol content and anthocyanin content depend on weather conditions. This could explain the differences between the berries picked in 2019 and 2020, and thus also differences between the reports as Centria only analyzed berries from 2020.

LAMMC observed differences in the anthocyanin profile of bilberries. In bilberries from Norway and Finland, the major anthocyanins were Dp-3-ara, followed by Dp-3-gal and Dp-3-glc. In bilberries from Latvia and Lithuania, the major anthocyanins were Cy-3-glc, followed by Cy-3-gal and Dp-3-glc. Bilberries from Latvia and Lithuania also had considerably lower Dp-3-gal and Dp-3-glc contents but higher level of Pn-3-glc than bilberries from Norway and Finland. Thus, some differences in the origin of bilberries can be identified with anthocyanin profile analysis.

Polyphenol content and antioxidant capacity were typically higher in berries from northern latitudes. However, chemical content of berries is affected by many factors and the natural variations in the chemical content can be substantial even inside the regions. Thus, quality verification is important regardless of the origin. Both LAMMC and Centria found correlation between antioxidant capacity and polyphenol content. In bilberries, lower correlation was found between antioxidant capacity and anthocyanin content. However, with lingonberries correlation between anthocyanins and antioxidants were not detected.

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