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O4.3 Berry lipid quality and authentication results obtained from bilberries and lingonberries picked 2019 and 2020 from several regions

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Abstract

Forest berries and their products are gaining expanded popularity due to their better taste, natural origin, and health benefits. However, there is widespread lying about the origin of these berries in recent years so that lower-quality berries can be sold at a higher price. A specific focus was to identify lingonberry (*Vaccinium vitis-idaea*) and bilberry (*Vaccinium myrtillus L.*) samples from 4 different countries in a two-year period. To establish an accurate method for identifying the origin country of the berries by their lipid composition, the gas chromatography-mass spectrometry (GC-MS) approach was adopted. The used method may authenticate the lipid origin of blueberries due to a good correlation between the growth site and the years.

Contents

1. Introduction

Today, the opportunity to transport fruits and vegetables increases, allowing us to enjoy diversity on our menu. To achieve the objective, a variety of procedures are in place to prevent products from deteriorating. Such practices reduce the nutritional value of products, which also leads to lower prices. Fruit, vegetables, and berries imported into the country are often marketed as local products, with prices consistently significantly higher than imported products. This kind of activity deceives buyers by making them overpay for more defective products [1]. Also, increased logging has reduced forest areas where it is possible to obtain forest-growing berries, mushrooms, and other raw materials. Due to such circumstances, products may be obtained in prohibited regions where there is a high level of environmental pollution. Plants from the contaminated area host nutrients together with possible contamination. If these plants are used in food, then it is possible to accumulate ecological pollution. Using authentication methods, food services and the buyer could track the true origin of food, reducing the number of unfair transactions and limit the uptake of environmental contaminants.

2. Materials and Methods

2.1 Samples

Harvested berries were purified, sorted and frozen at -20℃ degrees. Berries from other countries were frozen and transported in dry ice. Bilberry and lingonberry lipid extracts were prepared using $CHCl_3$ (Sigma-Aldrich, Germany) and filtered with Grade 1 Qualitative filter paper (Whatman, USA). Samples for GC analysis were dissolved in pyridine and derivatized using BSTFA (Sigma-Aldrich). The reaction was performed in 2 mL GC vials (Waters, USA). Quantification of compounds was done using standard solutions of ergosterol (≥99.0%), 1-octadecanol (≥99.0%), dodecanal (≥98.0%), stearic acid (≥98.5%), benzoic acid (≥99,5%) and n-tetracosane (≥99.5%) obtained from Sigma-Aldrich, and ursolic acid (HPLC ≥98%) obtained from Extrasynthese, standards were prepared in the concentration range of 3–800 μg/ml.

Two wild berries growing in Latvia, Lithuania, Norway, and Finland year 2019 and 2020 were investigated: lingonberries (*Vaccinium vitis-idaea* L.) and bilberries (*Vaccinium myrtillus* L.). The samples were analysed in three iterations.

2.2 Extraction

The extraction of berry lipids 30-50 g of selected berries was freeze-dried and homogenised in a sample grinder (IKA, Germany). 3-15 g of homogenised berries were weighed in 100 mL bottles with a cap and mixed with 70 mL of $CHCl_3$, then placed in an ultrasound bath for 20 min (Cole-Parmer, USA). The sonicated sample was then filtered through a filter paper. The used paper filter with berry particles was placed back into the extraction bottle and added another 70 mL of $CHCl_3$. Filtration and re-extraction in ultrasound were repeated three times. The fourth extraction was performed by incubating the sample in $CHCl_3$ overnight at room temperature to increase the extraction yield. The water in the ultrasound bath was changed every 10 min to avoid the evaporation of $CHCl_3$ and overheating.

After extraction, all the extracts were filtered, combined, and concentrated using a rotary evaporator (Heidolph, Germany). After evaporation, the berry lipids were dried under a stream of nitrogen (AGA, Latvia), and the dry samples were weighed and stored at 4℃.

2.3 Analysis

Extracted berry lipids and lipid fractions were weighed out (bilberry approx. 30 mg, lingonberries approx. 10 mg) in a GC vial and dissolved in 1.3 mL pyridine, and 0.2 mL of BSTFA was added. The sample was then heated at 60℃ for 1 h. The resulting sample was analysed using GC-MS.

GC-MS analysis were performed using GC - 2010 plus, GC - MS QP - 2010 Ultra gas chromatography and mass detector (Shimadzu, Japan). Rxi ® - 5MS (Restek, USA) column was used with a temperature range of 40−350 ℃. Helium (AGA, Latvia) was used as a carrier gas with a flow rate of 16.0 mL/min, a column flow rate of 1.18 mL/min, a pressure of 77.8 kPa, and a column purge flow rate of 3.0 mL/min. A split ratio of 1:10 and an injection temperature of 290℃ was used, temperature settings at the time of analysis: the initial temperature of the oven 75.00 ℃ was held for 2.00 min, increased from 75℃ to 130 ℃ with a rate of 20℃/min, held for 10.00 min, increased from 130℃ to 310℃ with the rate of 4 ℃/min and held at 310℃ for 10 min. Total runtime - 70 min. Injection volume 1 µL. Mass analyser electron impact was set to 70 eV, detector gain of 0.98 kV + 0.40 kV, scan mode was used with event time 0.30 sec, scan speed 2,500, from m/z 35.00 to m/z 650.00. LabSolutions 4.30 software (Shimadzu, Japan) and NIST17 (NIST, USA) Spectral Library was used for identification and quantification of compounds. The analyses were performed in triplicate.

3. Results and discussion

Contains the quality results of bilberry and lingonberry picked in Finland (FI), Norway (N), Latvia (LV) and Lithuania (LT) 2019 and 2020.

3.1 Analysis of total extracts

For the analysis of the extracts, gas-liquid chromatography with mass spectrometric detection was used. The lipid samples of cranberry were more yellowish paste, but the blueberry lipid samples were green and oilier. Oiliness could be due to higher concentrations of triglycerides in blueberry lipids. The total extracts revealed 34-39 compounds in lingonberries and 43-45 compounds in blueberries.

3.2 Analysis of lingonberry extracts

Most compounds in total lingonberry extracts are composed of triterpenes (53.6-73.5%), fatty acids (7.6-17.0%) and unidentified compounds (4.2-4.9%) (Figure 2.1.). Ursolic acid (75.15 to 347.10 mg/g of extract) was found among triterpenes at the highest concentration. The amount of triterpenes in 2019 and 2020 in Latvia (135 and 310 mg/g of extract), Lithuania (170 and 180 mg/g of extract), Finland (250 and 326 mg/g of extract) and Norway (77 and 180 mg/g of extract) is different (Table 2.1. and 2.2.). Growth conditions may explain the difference in extracts.

The extract data obtained were compared after years (Figure 2.2), where it can be seen that conditions in 2019 had a positive effect on the formation of fatty acids and sterols, an increase in 2020 compared to 2019 was observed in the amount of triterpenes and unidentified compounds in lipid extract. Other conditions have not significantly affected the formation of alcohols, aldehydes, and alkanes in lingonberry lipids.

The lingonberry lipid extracts were analysed using the JMP statistic 16 program's PCA analysis method, comparing the resulting data between the composition of the extract, the composition of the combination groups and the year. In assessing the results obtained, there may be no correlation between lingonberry lipid extractives and their countries of origin, as they are in entertainment with each other. The potential causes of the difference in the results could be harvesting time, weather conditions, or preparation of samples.

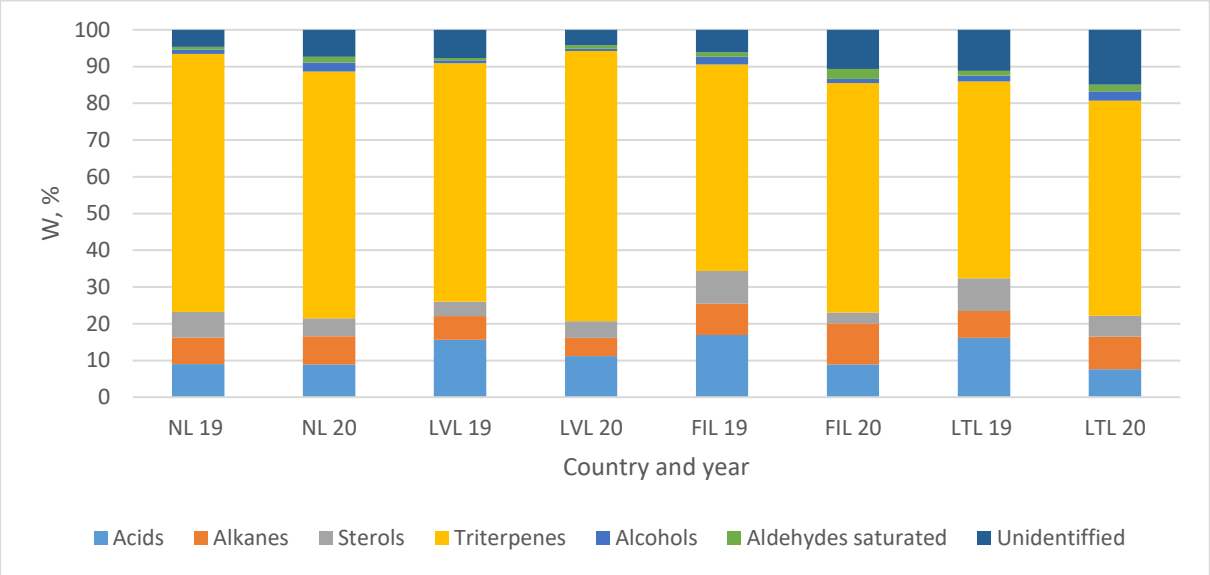

Figure 2.1. Percentages of detected compound classes in lingonberry lipid extract.

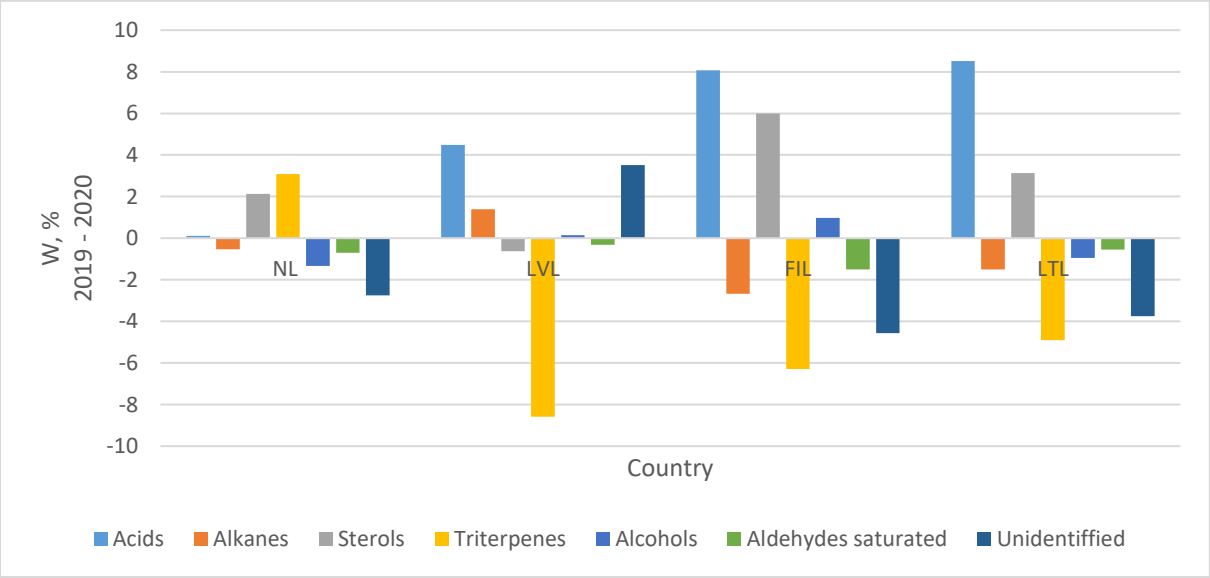

Figure 2.2. Change the percentage of lingonberry lipid extract between years.

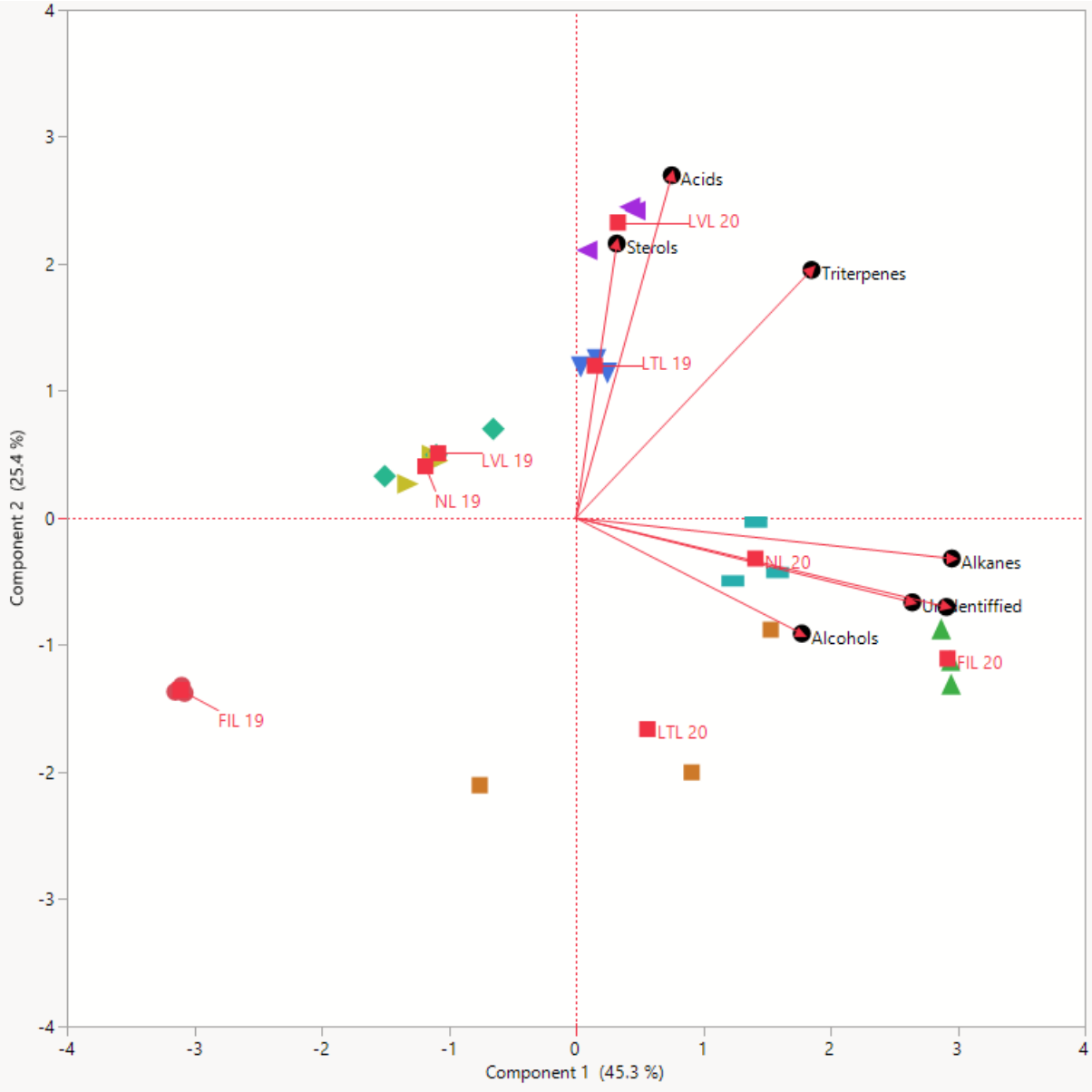

Figure 2.3. PCA analysis of lingonberry lipids.

3.3 Analysis of bilberry extracts

Most compounds in total bilberry extracts are composed of fatty acids (22.6-46.6%), triterpenes (13.9-40.0%) and sterols (14.1-33.2%) (Figure 2.4.). The citric acid (2.8-44.0 mg/g of extract), oleanolic acid (8.4-44.4 mg/g of extract) and b-sitosterol (34.2-79.2 mg/g of extract) was found among fatty acids, triterpenes, and sterols at the highest concentration.

The amount of triterpenes in 2019 and 2020 in Latvia, (135 and 310 mg/g of extract), Lithuania (170 and 180 mg/g of extract), Finland (250 and 326 mg/g of extract) and Norway (77 and 180 mg/g of extract) is different (Table 2.3. and 2.4.).

If we compare lipid data between 2019 and 2020, then quantitative proportions of compound groups are distinguishable. In 2019 fatty acids (2.2-6.9%) have formed more, while triterpenes (3.1-5.4%), alkanes (0-1.7%) and alcohols (0-1.4%) have formed more in 2020. The levels of sterols (0.4-8.8%) and aldehydes (0.1-2.5%) in blueberry lipids have changed significantly in different countries (Figure 2.5.).

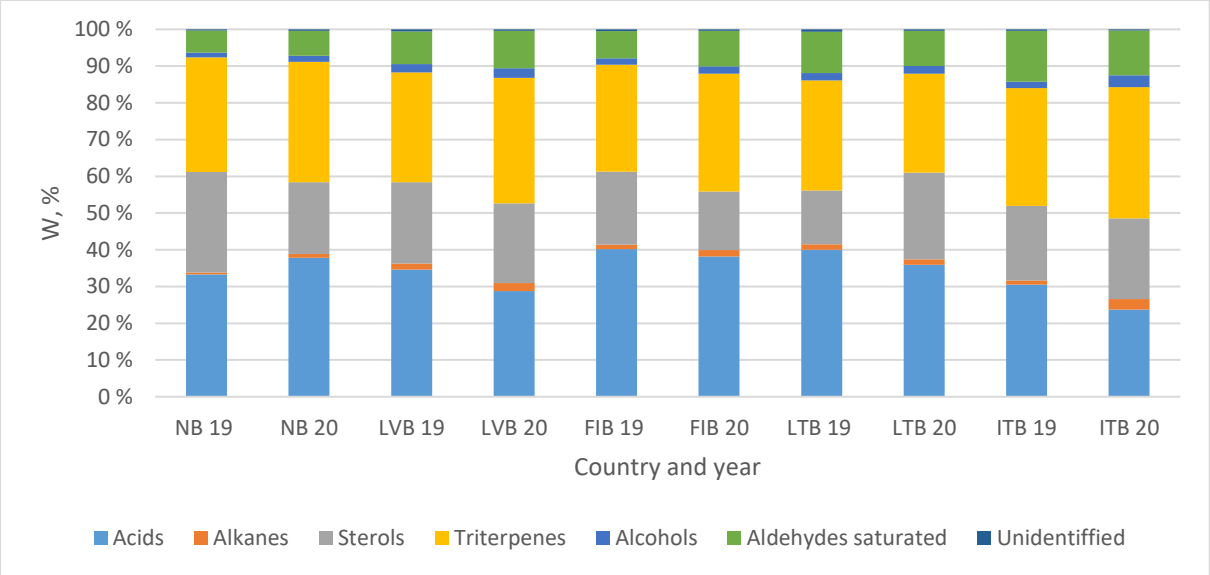

Figure 2.4. Percentages of detected compound classes in bilberry lipid extract.

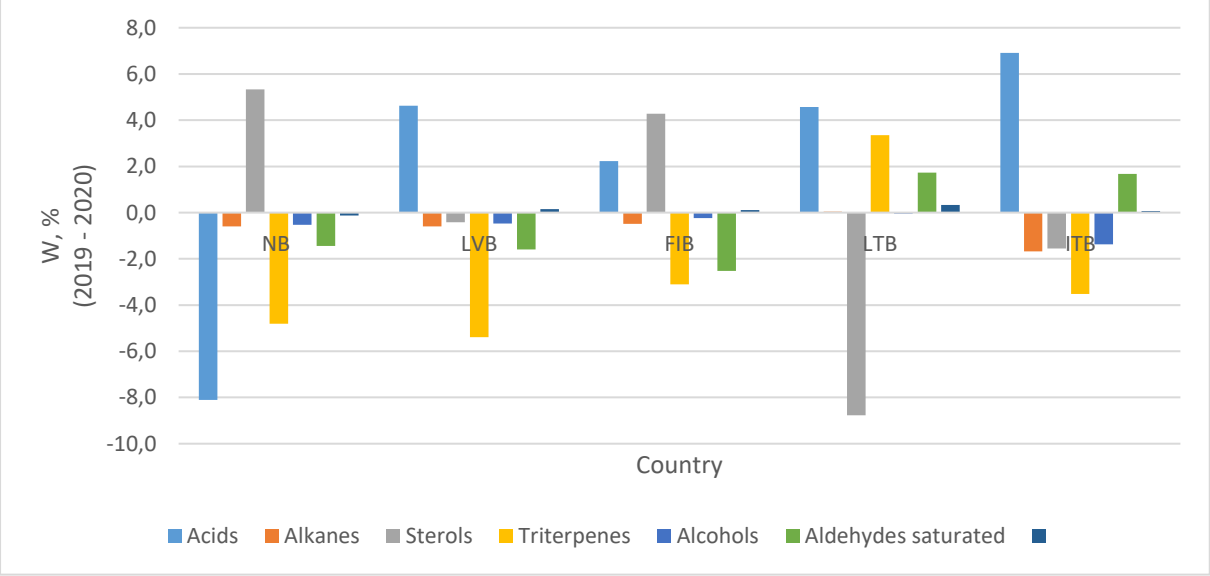

Figure 2.5. Change the percentage of bilberry lipid extract between years (2019 year up, 2020 year down).

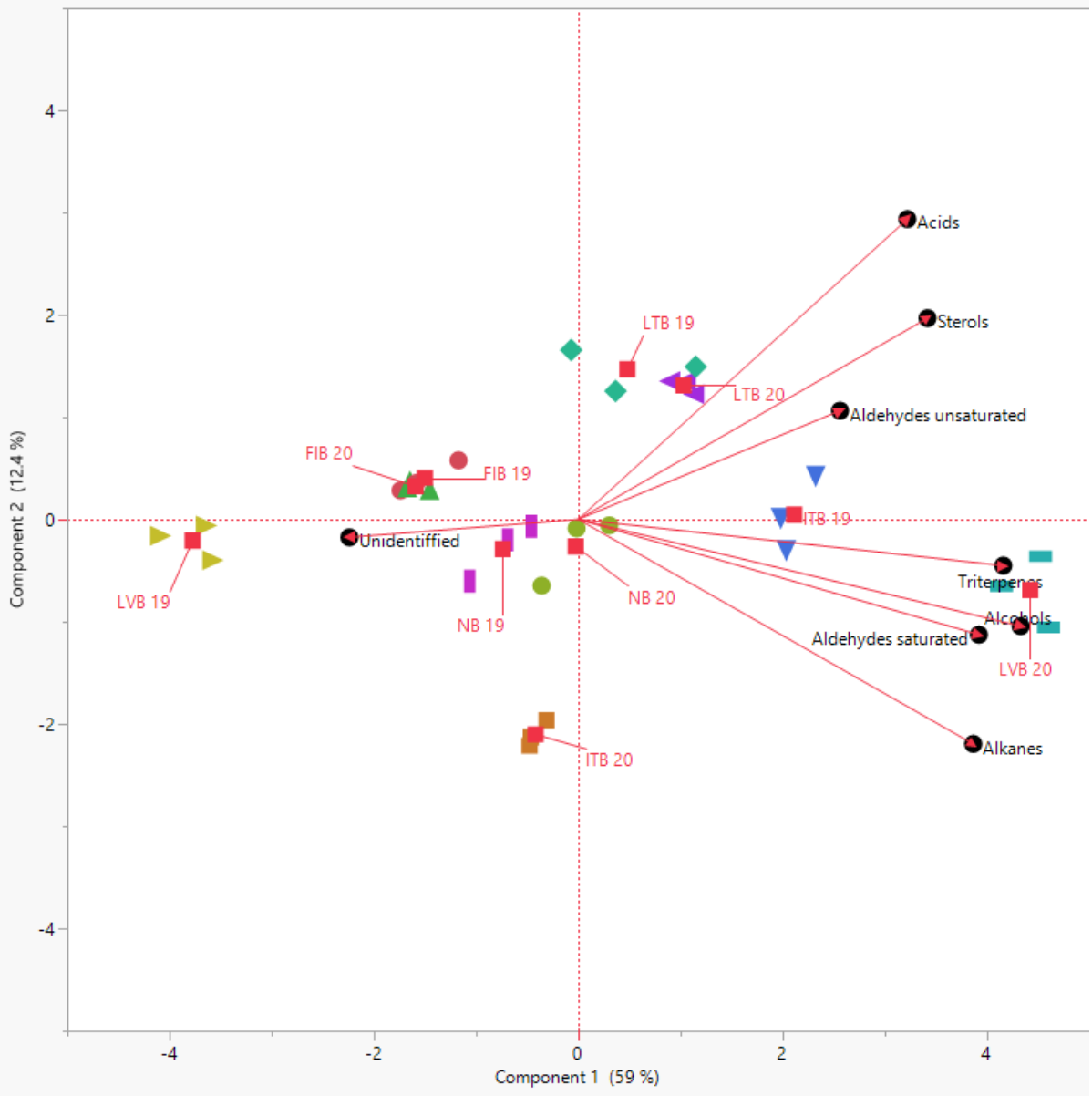

Figure 2.6. PCA analysis of bilberry lipids.

The bilberry lipid extracts were analysed using the PCA analysis method, comparing the resulting data between the extract's composition and the year. In assessing the results obtained, there are correlations between Norwegian (NB), Finnish (FIB) and Lithuanian (LTB) samples, depending on the location of experimental data points between years (Figure 2.6.). Similar data results show that it is possible to compare and identify the origin of berries harvested in one place, regardless of the year. As for Italian and Latvian samples, there is no correlation between the years. Possibly berries have been gathered in different locations, or there have been logging jobs in their natural growth site, which increased the intensity of the Sun and water by altering the lipid chemical composition and the possibility of obtaining a correlation between the years.

3.3 Other authentication methods

New methods are currently being developed in laboratories to identify the origin of the products. The most common methods are inductively conducted plasma atomic emission spectrometry (ICP-AES), isotope ratio mass spectrometry (IRMS) and inductively conducted plasma mass spectrometry (ICP-MS). These methods determine the bio cell isotope ratio found in the biological material (hydrogen [$d^{2}H$], carbon [$d^{13}C$], nitrogen [$d^{15}N$], oxygen [$d^{18}O$], sulfur [$d^{32}S$]) and heavy-element isotopes (lead [$d^{207}Pb$], strontium [$d^{87}Sr$]) [2]. The ratio of the light element isotopes represents agricultural practices, environmental conditions (light cycles and intensity, temperature, humidity, and sun radiation). The ratio of the heavy element isotopes provides information on the geological composition of the location[3].

Using lipid analyses, ICP-MS and weather data could assess the lipid composition of berries depending on weather conditions. If there were a correlation between the data collected, it would be possible to create a database by which the analysis of berry lipids could determine the country of origin.

4. Summary

For the determination of berry origin, it is necessary to develop a reliable authentication method based on changes in the chemical or quantitative composition of berries, depending on the place of growth. The study was based on the chemical and quantitative exploration of berry lipid extract between 4-5 different countries over two years. The statistically analysed lipid samples of lingonberries did not produce a correlation between growth countries and years (Figure 2.3.) as data were distributed. On the other hand, bilberry lipid samples made a good correlation between the growth site and the years. Latvia's representative samples were on the opposite sides of the horizontal line, and Italian samples were diagonal (Figure 2.6.).

In evaluating the results obtained, the overall lipid analysis could be used as berry growth site authentication for blueberry samples taken together with measurements of stable isotope ratios based on the lipid biosynthesis pathway dependency on environmental conditions.

Table 2.1. 2019 cranberry lipid composition.

Acids														
Ret time	Ret. Ind. Practical	Ret. Ind. Literature	Name	mg/100 g dry weight (DW)											
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
7.188	1247	1253	**Benzoic Acid, TMS**	9.50	9.92	9.91	9.39	9.46	9.54	19.36	20.15	20.82	20.30	23.38	22.80
7.737	1279	N/A	**Pphosphate (3:1), TMS**	3.65	3.97	4.22	2.19	2.09	2.13	3.83	4.55	4.41	2.69	2.82	2.17
17.378	1546	1547	**Cinnamic acid, (E)-, TMS**	–	–	–	–	–	–	–	–	–	1.85	1.89	1.84
27.247	1805	1789	**Azelaic acid, 2TMS**	1.20	1.27	1.23	0.97	0.89	0.82	2.87	2.48	2.23	3.83	3.04	2.52
28.640	1851	1853	**Citric acid, 4TMS**	3.07	3.49	3.18	UQL	UQL	UQL	3.83	4.23	4.42	7.63	8.28	7.52
29.970	1896	1863	**Quininic acid, 5TMS**	6.90	7.77	7.55	3.99	3.97	4.15	6.96	7.13	7.50	8.07	8.75	8.04
34.052	2048	2043	**Palmitic Acid, TMS**	3.36	3.59	3.61	1.58	1.54	1.59	3.47	3.42	3.30	3.87	2.83	2.41
38.019	2212	2215	**9-Octadecenoic acid, (E)-, TMS**	4.41	4.73	4.72	4.45	4.43	4.49	10.28	10.30	10.22	8.55	6.52	6.09
38.160	2218	2219	**α-Linolenic acid, TMS**	4.26	4.43	4.77	5.25	5.22	5.73	11.89	11.73	11.38	15.08	10.38	9.12
38.772	2245	2240	**Stearic acid, TMS**	–	–	–	–	–	–	–	–	–	1.23	1.00	0.91
46.993	2640	2638	**Docosanoic acid, TMS**	1.33	1.44	1.49	1.57	1.56	1.58	2.43	2.48	2.49	1.84	1.67	1.59
50.643	2837	2837	**Tetracosanic acid, TMS**	2.36	2.62	2.66	2.17	2.20	2.21	4.26	4.28	4.33	2.97	2.68	2.49
Alkanes															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
44.277	2501	2500	**Pentacosane**	0.28	0.43	0.44	0.19	0.16	0.20	3.43	3.37	3.32	0.56	0.19	0.05
48.114	2698	2700	**Heptacosane**	30.77	32.54	32.82	17.52	17.18	17.53	32.84	33.06	33.15	33.74	31.37	28.46
49.904	2795	2800	**Octacosane**	0.19	0.20	0.25	UQL	UQL	UQL	UQL	UQL	UQL	UQL	UQL	UQL
51.711	2897	2900	**Nonacosane**	3.70	4.01	3.90	0.54	0.28	0.33	0.32	0.40	0.34	1.63	1.36	1.08
53.444	2998	3000	**Triacontane**	UQL	UQL	UQL	0.11	0.08	0.15	UQL	UQL	UQL	0.13	UQL	UQL
Sterols															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3

59.327	3368	3356	**b-Sitosterol, TMS**	34.56	35.88	35.99	19.16	18.76	19.34	44.71	43.81	43.81	21.02	19.91	18.94
Triterpenes															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
59.498	3380	3385	**β-Amyrin, TMS**	15.61	15.74	16.22	8.84	8.80	9.07	16.11	16.61	16.45	21.93	21.89	21.14
60.059	3416	3429	**α-Amyrin, TMS**	39.53	41.55	41.54	23.32	23.54	22.96	29.72	29.15	28.93	33.33	31.61	30.20
60.190	3424	3434	**Lupeol, TMS**	35.08	–	–	–	–	–	25.88	25.13	26.52	100.18	97.61	91.86
62.514	3562	N/A	**Uvaol, 2TMS**	–	–	–	–		–	–	–	–	25.71	22.50	19.43
63.214	3601	N/A	**Oleanolic acid, 2TMS**	35.07	37.01	36.70	10.25	10.97	6.96	22.65	24.03	26.31	24.76	22.20	22.24
64.136	3644	3657	**Ursolic acid, 2TMS**	238.30	255.90	260.11	75.15	77.36	82.31	160.99	171.53	183.10	135.55	135.24	136.91
Alcohols															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
45.311	2553	2554	**1-Docosanol, TMS**	–	–	–	0.41	0.40	0.41	1.02	1.07	1.07	0.43	0.32	0.27
49.049	2749	N/A	**1-Tetracosanol, TMS**	0.31	0.31	0.34	0.67	0.65	0.66	1.16	1.22	1.16	0.49	0.43	0.34
52.552	2946	2950	**1-Hexacosanol, TMS**	1.81	1.94	1.79	1.76	1.63	1.74	3.10	3.19	3.22	1.53	1.31	1.16
54.216	3044	3037	**1-Heptacosanol, TMS**	0.88	1.00	1.04	0.55	0.54	0.59	0.82	0.90	0.93	0.55	0.50	0.39
55.823	3143	3154	**1-Octacosanol, TMS**	1.13	1.16	1.15	0.81	0.64	0.79	1.09	1.73	1.49	1.08	1.45	1.00
57.422	3244	N/A	**1-Nonacosanol, TMS**	1.20	1.30	1.28	0.36	0.42	0.38	0.30	0.30	0.37	0.37	0.36	0.35
Aldehydes saturated															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
46.837	2631	2632	**Tetracosanal**	UQL	UQL	UQL	UQL	UQL	UQL	0.36	0.35	0.29	UQL	UQL	UQL
52.473	2941	2944	**Heptacosanal**	0.64	0.83	0.78	0.84	0.95	0.79	2.87	3.02	2.87	0.52	0.31	UQL
54.120	3039	3032	**Octacosanal**	3.22	3.48	3.44	1.73	1.67	1.59	3.18	3.47	3.10	3.28	2.94	2.64
Unidentiffied															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
58.974	3345	N/A	**?**	22.61	24.77	22.96	13.70	12.26	13.15	55.12	56.78	55.09	45.67	37.79	34.60

Table 2.2. 2020 cranberry lipid composition.

Acids														
Ret time	Ret. Ind. Practical	Ret. Ind. Literature	Name	mg/100 g DW										
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
7.188	1247	1253	**Benzoic acid, TMS derivative**	1.07	0.82	0.94	27.47	28.48	28.17	0.92	0.63	0.86	27.36	27.81	27.90
7.737	1279	N/A	**Pphosphate (3:1), TMS**	3.94	4.28	4.56	2.10	2.01	2.05	1.91	2.26	2.19	1.97	2.07	1.59
17.378	1546	1547	**Cinnamic acid, (E)-, TMS**	–	–	–	–	–	–	–	–	–	1.36	1.39	1.35
27.247	1805	1789	**Azelaic acid, 2TMS**	0.62	0.57	0.59	0.81	0.87	0.86	0.35	0.31	0.34	0.97	0.99	1.02
28.640	1851	1853	**Citric acid, 4TMS**	0.78	0.80	0.82	UQL	UQL	UQL	0.79	0.66	0.82	1.21	1.08	1.09
29.970	1896	1863	**Quininic acid, 5TMS**	–	16.11	–	7.24	8.08	–	17.81	–	–	22.20	22.39	22.65
34.052	2048	2043	**Palmitic acid, TMS**	22.21	21.25	21.48	7.33	8.25	7.67	15.09	10.67	14.69	12.74	13.01	13.23
38.019	2212	2215	**9-Octadecenoic acid, (E)-, TMS**	1.62	1.52	1.70	1.14	1.22	1.22	1.08	0.81	1.07	1.77	1.61	1.63
38.160	2218	2219	**α-Linolenic acid, TMS**	1.60	1.62	1.65	0.97	1.04	1.03	1.07	0.77	1.13	2.58	2.57	2.55
38.772	2245	2240	**Stearic acid, TMS**	0.89	0.89	0.90	0.51	0.55	0.54	0.63	0.49	0.59	0.77	0.78	0.79
43.051	2440	2437	**Arachidic acid, TMS**	–	–	1.45	0.79	0.82	0.84	0.61	0.44	0.53	0.81	0.74	0.75
46.993	2640	2638	**Docosanoic acid, TMS**	5.35	5.36	5.54	2.42	2.69	2.72	2.57	1.68	2.26	1.05	1.02	1.07
50.643	2837	2837	**Tetracosanic acid, TMS**	6.85	6.83	7.12	4.00	4.61	4.66	4.40	2.21	3.36	2.19	2.30	2.36
57.280	3235	3233	**Octacosanoic acid, TMS**	1.41	1.49	1.49	1.03	1.06	1.08	1.28	0.69	0.81	1.72	1.68	1.69
60.302	3431	3426	**Triacontanoic acid, TMS**	–	–	–	–	–	–	–	–	–	2.95	2.80	2.76

Alkanes															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
40.030	2301	2300	**Tricosane**	1.73	1.75	1.80	3.00	3.17	3.00	0.72	0.22	0.74	0.23	0.21	0.22
44.277	2501	2500	**Pentacosane**	41.69	41.18	42.75	60.45	62.01	61.72	35.46	27.21	36.49	35.30	34.23	34.32
48.114	2698	2700	**Heptacosane**	UQL	UQL	UQL	0.60	0.62	0.54	0.39	UQL	0.23	UQL	UQL	UQL
49.904	2795	2800	**Octacosane**	2.59	2.61	2.62	4.70	4.96	5.06	5.73	4.46	5.68	4.48	4.30	4.71
51.711	2897	2900	**Nonacosane**	2.52	2.29	2.51	2.32	2.17	2.37	UQL	UQL	0.21	0.05	0.20	0.33
Sterols															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
59.327	3368	3356	**β-Sitosterol, TMS**	30.75	30.32	30.70	18.56	19.56	19.54	28.54	20.73	26.72	35.35	35.97	35.67
Triterpenes															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
59.498	3380	3385	**β-Amyrin, TMS**	15.75	16.43	16.64	88.73	95.10	93.56	6.47	5.37	6.68	20.94	21.53	21.63
60.059	3416	3429	**α-Amyrin, TMS**	25.32	26.31	25.89	53.06	56.57	55.86	16.66	12.03	16.46	28.88	28.22	29.77
60.190	3424	3434	**Lupeol, TMS**	2.65	1.80	1.80	35.50	37.67	37.77	8.43	5.51	8.03	175.62	174.84	174.77
61.175	3486	N/A	**Glutinol, TMS**	–	–	–	–	–	–	–	–	–	20.47	20.71	19.71
62.514	3562	N/A	**Uvaol, 2TMS**	11.31	11.42	11.46	12.58	13.46	12.68	7.25	6.61	7.53	18.23	18.12	20.96
63.214	3601	N/A	**Oleanolic acid, 2TMS**	34.61	36.23	38.31	22.02	27.83	29.29	28.06	14.22	14.26	41.78	43.14	41.39
64.136	3644	3657	**Ursolic acid, 2TMS**	316.18	326.64	347.10	160.67	181.75	203.17	295.47	166.69	173.56	316.27	201.35	305.91
Alcohols															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
45.311	2553	2554	**1-Docosanol, TMS**	0.99	0.98	0.89	0.61	0.65	0.66	1.78	1.11	1.75	0.12	0.09	0.11
49.049	2749	N/A	**1-Tetracosanol, TMS**	2.36	2.37	2.45	0.72	0.78	0.73	2.84	1.84	2.67	0.23	0.28	0.25
52.552	2946	2950	**1-Hexacosanol, TMS**	6.04	5.89	6.20	1.86	2.00	1.97	4.54	3.03	4.36	1.56	1.56	1.58
54.216	3044	3037	**1-Heptacosanol, TMS**	1.91	2.12	2.27	0.85	0.91	0.94	1.49	0.83	1.42	0.82	0.80	0.88
55.823	3143	3154	**1-OLtacosanol, TMS**	1.96	2.09	2.24	3.35	1.79	2.05	1.41	1.02	1.40	1.42	1.25	1.24
57.422	3244	N/A	**1-Nonacosanol, TMS**	1.54	1.63	1.79	0.64	0.56	0.63	1.12	0.83	0.97	1.13	1.21	1.04

Aldehydes saturated															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
42.786	2427	2430	**Docosanal**	1.19	1.26	1.38	1.08	0.95	0.99	0.40	UQL	0.46	UQL	UQL	UQL
46.837	2631	2632	**Tetracosanal**	1.18	1.16	1.28	2.04	1.79	1.85	0.62	0.07	0.65	UQL	UQL	UQL
52.473	2941	2944	**Heptacosanal**	3.56	3.50	3.85	5.44	4.66	4.94	2.53	1.24	2.24	1.44	1.23	1.37
54.120	3039	3032	**Octacosanal**	3.02	3.55	3.70	10.23	8.99	9.24	6.17	4.27	6.40	6.38	5.85	6.19
Unidentiffied															
				NLX1	NLX2	NLX3	FILX1	FILX2	FILX3	LTLX1	LTLX2	LTLX3	LVLX1	LVLX2	LVLX3
58.974	3345	N/A	**?**	46.29	46.49	46.08	66.29	70.79	70.65	74.45	54.96	68.90	32.01	33.06	33.27

Table 2.3. 2019 blueberry lipid composition.

Acids																	
Ret time	Ret. Ind. Practical	Ret. Ind. Literature	Name	mg/ 100 g DW													
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
4.857	1077	1071	**Hexanoic acid, TMS**	5.92	5.83	6.07	1.55	1.56	1.51	1.04	1.03	1.08	1.12	2.09	2.13	0.95	0.95	0.95
4.911	1082	1077	**Glycolic acid, 2TMS**	2.05	2.02	2.15	0.77	0.75	1.51	0.62	0.60	0.62	0.49	0.75	0.73	0.56	0.56	0.56
6.829	1226	N/A	**Propanoic acid, 2TMS**	1.46	1.51	1.47	0.70	0.71	0.69	0.52	0.53	0.54	0.42	0.56	0.56	0.44	0.43	0.43
7.188	1247	1253	**Benzoic acid, TMS**	–	–	–	0.62	0.62	0.62	–	–	–	–	0.37	0.39	0.33	–	–
7.737	1279	N/A	**Phosphate (3:1), TMS**	10.17	9.58	9.36	7.65	9.44	9.77	8.01	10.01	10.59	2.66	5.08	5.69	5.29	6.43	6.31
9.277	1344	1345	**2-Butenedioic acid, (E)-, 2TMS**	1.72	1.62	1.67	0.60	0.58	0.45	0.61	0.52	0.45	0.46	0.65	0.62	0.33	0.37	0.46
15.467	1505	N/A	**Malic acid, 3TMS**	2.59	2.61	2.54	4.45	4.74	4.67	5.69	5.33	5.58	1.24	1.77	1.75	1.74	1.73	1.78
17.378	1546	1547	**Cinnamic acid, (E), TMS**	UQL	UQL	UQL	–	–	–	–	–	0.31	0.33	–	–	0.25	0.28	0.28
27.247	1805	1789	**Azelaic acid, 2TMS**	4.19	7.39	9.20	6.64	4.24	3.69	1.27	2.16	2.37	1.96	3.08	3.35	4.79	3.68	3.00
28.640	1851	1853	**Citric acid, 4TMS**	5.51	5.75	4.75	25.26	28.05	–	43.54	42.30	44.01	2.83	3.38	2.76	17.43	4.09	18.22
29.970	1896	1863	**Quininic acid, 5TMS**	6.14	6.32	6.28	8.33	9.11	8.99	8.02	8.01	8.37	1.72	2.07	2.08	2.95	2.90	2.93
34.052	2048	2043	**Palmitic acid, TMS**	6.54	6.92	7.75	5.54	5.42	6.28	6.58	6.55	0.45	3.93	4.45	4.27	6.81	5.96	6.64
38.019	2212	2215	**9-Octadecenoic acid, (E)-, TMS**	12.18	12.34	14.58	8.49	8.15	8.17	14.61	14.52	14.75	7.50	8.24	8.02	11.85	11.56	11.57
38.160	2218	2219	**α-Linolenic acid, TMS**	21.37	23.87	27.45	13.33	13.29	13.21	19.66	1.20	19.13	11.78	12.70	12.60	18.07	16.77	17.06
38.772	2245	2240	**Stearic acid, TMS**	2.23	2.20	2.45	1.81	1.63	1.57	1.99	1.76	2.00	1.08	1.16	1.13	1.94	0.44	1.88
43.051	2440	2437	**Arachidic acid, TMS**	1.35	1.38	1.42	1.31	1.24	1.28	1.22	1.11	1.20	0.73	0.76	0.77	0.91	0.96	0.89

46.993	2640	2638	**Docosanoic acid, TMS**	0.92	0.99	1.00	0.92	0.95	0.93	–	0.37	–	0.52	–	0.51	0.25	0.65	0.28
50.643	2837	2837	**Tetracosanic acid, TMS**	1.25	1.20	1.18	1.56	13.71	13.61	–	1.20	–	0.85	–	0.57	–	0.93	–
57.280	3235	3233	**Octacosanoic acid, TMS**	5.11	5.10	4.92	10.27	10.86	11.01	6.70	6.65	7.27	1.59	2.03	2.01	6.32	6.42	6.60
60.302	3431	3426	**Triacontanoic acid, TMS**	1.23	1.49	1.35	UQL	1.94	1.94	–	2.14	2.33	–	–	0.51	3.29	3.46	3.41
Alkanes																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
40.030	2301	2300	**Tricosane**	0.83	0.89	0.79	1.52	1.56	1.57	0.81	0.78	0.81	0.20	0.31	0.29	0.34	0.33	0.33
44.277	2501	2500	**Pentacosane**	UQL	UQL	UQL	0.77	0.75	0.85	0.20	0.21	0.24	UQL	UQL	UQL	0.15	0.29	0.19
48.114	2698	2700	**Heptacosane**	0.59	0.48	0.77	0.52	0.56	0.60	1.47	1.53	1.27	UQL	UQL	UQL	1.81	2.02	1.90
53.444	2998	3000	**Triacontane**	–	UQL	–	1.28	0.79	0.67	–	–	–	–	–	UQL	UQL	–	–
Sterols																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
59.327	3368	3356	**β-Sitosterol, TMS**	48.15	48.94	48.68	68.87	67.32	66.93	75.35	75.89	76.35	39.44	44.01	42.82	49.45	48.00	48.24
Triterpenes																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
59.498	3380	3385	**β-Amyrin, TMS**	10.25	10.99	10.59	15.68	15.18	15.44	18.98	18.46	20.26	8.22	10.21	9.40	19.41	19.02	18.85
60.059	3416	3429	**α-Amyrin, TMS**	3.95	4.12	3.76	7.05	7.16	6.70	6.52	5.61	5.69	6.04	2.38	7.32	8.65	8.07	8.05
60.190	3424	3434	**Lupeol,TMS**	–	–	–	3.95	–	3.76	–	11.23	11.81	3.49	–	3.51	16.95	–	17.54
61.175	3486	N/A	**Glutinol, TMS**	6.13	5.80	5.55	1.30	12.85	10.78	5.53	6.16	5.88	1.32	3.37		2.54	3.22	–
62.514	3562	N/A	**Uvaol, 2TMS**	–	4.50	3.18	1.47	–		2.24	–	3.16	–	–	–	8.62	8.49	4.59
63.214	3601	N/A	**Oleanolic acid, 2TMS**	26.87	27.18	23.93	36.85	41.13	44.36	–	27.62	29.04	5.30	8.77	8.39	24.88	24.54	26.98
64.136	3644	3657	**Ursolic acid, 2TMS**	–	–	2.06	30.81	33.22	33.13	2.63	2.64	27.58	5.47	9.17	8.61	24.35	23.92	25.29
Alcohols																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
45.311	2553	2554	**1-Docosanol, TMS**	0.07	0.10	0.11	0.15	0.18	0.18	0.21	0.21	0.17	UQL	UQL	UQL	0.01	0.02	0.00
49.049	2749	N/A	**1-Tetracosanol, TMS**	UQL	0.03	0.05	0.19	0.23	0.19	–	–	–	UQL	–	UQL	–	UQL	0.02

52.552	2946	2950	**1-Hexacosanol, TMS**	0.46	0.43	0.48	1.31	1.40	1.36	1.03	1.04	1.07	0.25	0.21	0.25	0.66	0.64	0.65
54.216	3044	3037	**1-Heptacosanol, TMS**	1.13	1.16	1.15	2.27	2.32	2.30	1.86	1.88	1.95	0.63	0.70	0.72	1.11	1.11	1.09
55.823	3143	3154	**1-Octacosanol, TMS**	1.03	2.24	1.76	1.54	1.48	1.58	1.81	1.79	2.23	0.58	0.55	0.64	1.78	1.84	2.15
57.422	3244	N/A	**1-Nonacosanol, TMS**	0.22	0.12	0.24	0.49	0.44	0.41	0.49	0.45	0.49	0.07	0.03	0.03	0.58	0.75	0.59
Aldehydes saturated																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
46.837	2631	2632	**Tetracosanal**	4.14	4.35	4.28	10.47	11.60	11.54	–	–	2.68	1.77	2.09	1.83	1.63	1.64	1.58
52.473	2941	2944	**Heptacosanal**	12.75	12.91	12.64	28.95	29.36	29.23	12.02	11.89	12.24	4.92	5.75	5.70	9.46	9.88	9.40
54.120	3039	3032	**Octacosanal**	3.68	3.90	3.59	5.62	5.58	6.43	3.82	3.94	3.93	0.02	0.06	0.07	6.14	7.21	6.06
Aldehydes unsaturated																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
7.403	1260	1254	**2-Decenal, (Z)-**	–	–	–	1.17	1.21	1.17	0.65	0.70	0.75	–	0.44	0.44	0.41	0.50	0.49
Unidentified																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
58.974	3345	N/A	**?**	–	8.49	–	–	–	–	2.02	–	–	–	8.06	15.39	–	5.04	3.93

Table 2.4. 2020 blueberry lipid composition.

Acids																	
Ret time	Ret. Ind. Practical	Ret. Ind. Literature	Name	mg/ 100 g DW													
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
4.857	1077	1071	**Hexanoic acid, TMS**	3.01	2.96	2.95	0.66	0.69	0.68	2.25	2.22	2.18	1.63	1.62	1.61	3.54	3.53	3.54
4.911	1082	1077	**Glycolic acid, 2TMS**	1.05	1.01	0.95	0.66	0.69	0.68	0.87	0.84	0.82	0.93	0.93	0.95	1.26	1.26	1.27
6.829	1226	N/A	**Propanoic acid, 2TMS**	1.05	0.99	0.99	0.37	0.37	0.36	0.68	0.70	0.73	0.86	0.84	0.82	1.25	1.22	1.20
7.737	1279	N/A	**Phosphate (3:1), TMS**	8.05	7.81	8.53	2.78	2.26	2.37	6.68	5.78	6.23	10.34	10.37	10.56	9.13	9.52	9.16
9.277	1344	1345	**2-Butenedioic acid, (E)-, 2TMS**	0.79	0.77	0.75	0.28	0.30	0.29	0.57	0.57	0.58	0.60	0.62	0.52	0.89	0.87	0.82
15.467	1505	N/A	**Malic acid, 3TMS**	2.39	2.31	2.29	0.79	0.82	0.81	1.95	1.94	1.96	2.06	2.23	2.17	2.18	2.26	2.17
27.247	1805	1789	**Azelaic acid, 2TMS**	3.43	4.18	4.24	1.88	1.74	1.68	3.72	3.53	3.52	3.98	3.74	3.71	6.23	6.72	6.53
28.640	1851	1853	**Citric acid, 4TMS**	17.62	17.15	17.19	–	–	–	21.27	21.14	21.05	28.49	30.00	18.10	17.50	18.50	0.29
29.970	1896	1863	**Quininic acid, 5TMS**	3.88	3.72	3.73	3.60	3.84	3.59	4.36	4.29	4.39	6.15	6.50	6.14	7.61	8.13	7.49
34.052	2048	2043	**Palmitic acid, TMS derivative**	4.64	4.62	4.49	4.35	3.90	4.23	6.99	5.58	6.84	7.25	8.57	8.71	7.80	6.81	6.57
38.019	2212	2215	**9-Octadecenoic acid, (E)-, TMS**	4.91	4.61	4.54	4.25	4.32	4.20	9.97	10.37	10.21	8.42	8.52	8.46	5.56	6.16	5.71
38.160	2218	2219	**α-Linolenic acid, TMS**	12.47	12.40	11.77	8.52	8.86	8.71	17.90	19.26	20.05	14.60	15.16	15.32	13.73	14.49	13.04
38.772	2245	2240	**Stearic acid, TMS**	1.45	1.50	1.58	1.10	1.16	1.05	2.08	1.92	2.05	2.50	2.30	2.43	2.14	2.28	2.20
43.051	2440	2437	**Arachidic acid, TMS**	0.97	0.86	0.93	0.83	0.81	0.80	1.13	1.14	1.12	1.74	1.70	1.67	1.21	1.26	1.18
46.993	2640	2638	**Docosanoic acid, TMS**	0.64	0.58	0.58	0.59	0.58	0.57	0.71	0.66	0.74	0.90	0.93	0.93	0.85	0.90	0.84
50.643	2837	2837	**Tetracosanoic acid, TMS**	0.66	0.64	0.67	1.24	6.73	1.23	0.92	0.87	0.85	1.21	1.24	1.78	1.16	1.21	1.20

57.280	3235	3233	**Octacosanoic acid, TMS**	4.16	4.36	4.28	4.33	4.68	4.36	7.83	7.46	7.63	10.72	11.39	11.24	4.32	5.53	4.44
60.302	3431	3426	**Triacontanoic acid, TMS**	1.59	1.11	1.02		0.97	0.86	2.30	2.19	2.30	4.08	4.26	4.32	0.88	0.94	0.87
Alkanes																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
40.030	2301	2300	**Tricosane**	0.09	0.07	0.06	1.49	1.53	1.49	0.07	0.08	0.07	0.94	0.92	0.93	1.10	1.09	0.93
44.277	2501	2500	**Pentacosane**	1.55	1.44	1.47	3.13	3.07	3.16	2.08	2.04	2.06	4.19	4.10	4.13	1.91	1.92	1.74
48.114	2698	2700	**Heptacosane**	0.27	0.21	0.44	0.71	0.66	0.74	0.74	0.73	1.04	2.66	2.56	2.63	0.16	0.06	0.19
51.711	2897	2900	**Nonacosane**	UQL	UQL	UQL	UQL	UQL	0.15	UQL	UQL	UQL	0.26	0.48	0.60	UQL	UQL	UQL
53.444	2998	3000	**Triacontane**	UQL	UQL	–	0.09	0.08	–	–	–	–	–	–	1.14	–	0.87	0.61
Sterols																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
59.327	3368	3356	**b-Sitosterol, TMS**	41.42	41.04	40.07	41.67	42.75	41.57	66.68	68.23	67.86	75.53	77.92	77.57	35.03	35.15	34.23
Triterpenes																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
59.498	3380	3385	**β-Amyrin, TMS**	8.96	8.90	8.57	14.80	15.44	14.58	22.41	22.91	23.01	30.33	31.82	30.94	12.62	12.83	12.84
60.059	3416	3429	**α-Amyrin, TMS**	2.52	1.85	6.20	3.87	4.33	4.36	13.06	7.83	6.76	19.09	19.18	13.81	3.62	3.89	4.16
60.190	3424	3434	**Lupeol, TMS**	4.02	5.80	6.20	2.90	2.65	2.83	13.06	13.56	12.83	19.29	21.29	19.29	3.58	4.05	3.31
61.175	3486	N/A	**Glutinol, TMS**	–	–	–	2.73	–	1.51	1.57	–	–	–	–	–	–	–	–
62.038	3535	N/A	**Erythrodiol, 2TMS**	–	–	–	3.90	3.76	3.09	–	–	–	3.36	21.47	19.88	–	2.11	–
63.214	3601	N/A	**Oleanolic acid, 2TMS**	17.56	15.10	15.55	23.17	28.08	27.50	29.91	31.60	31.62	30.51	33.16	35.24	30.76	28.98	27.96
64.136	3644	3657	**Ursolic acid, 2TMS**	16.17	15.49	15.75	15.24	15.29	14.84	16.51	16.48	16.57	29.76	31.97	30.68	21.76	21.74	20.11
Alcohols																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
45.311	2553	2554	**1-Docosanol, TMS**	UQL	UQL	UQL	0.15	0.16	0.13	UQL	0.01	0.02	0.01	UQL	0.02	0.12	0.13	0.13
49.049	2749	N/A	**1-Tetracosanol, TMS**	UQL	UQL	UQL	0.19	0.21	0.19	0.03	0.01	0.00	0.20	0.18	0.21	0.27	0.24	0.21
52.552	2946	2950	**1-Hexacosanol, TMS**	0.35	0.32	0.34	1.07	1.15	1.06	0.67	0.66	0.78	1.32	1.35	1.39	1.01	1.01	0.93
54.216	3044	3037	**1-Heptacosanol, TMS**	0.93	1.10	1.09	2.24	2.36	2.28	1.89	1.64	1.89	3.24	3.21	3.25	1.93	1.96	1.85

55.823	3143	3154	**1-Octacosanol, TMS**	1.53	1.63	1.55	1.92	1.90	1.87	2.35	1.94	2.04	4.06	3.34	3.70	1.37	1.27	1.28
57.422	3244	N/A	**1-Nonacosanol, TMS**	0.33	0.34	0.37	0.47	0.44	0.48	0.61	0.58	0.44	0.90	0.88	0.91	0.36	0.34	0.36
Aldehydes saturated																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
46.837	2631	2632	**Tetracosanal**	1.78	1.74	1.71	5.26	5.40	5.20	2.22	2.13	2.23	5.33	5.40	5.50	7.22	8.07	7.78
50.618	2835	2834	**Hexacosanal**	0.06	0.01	0.03	0.66	0.60	0.63	0.18	0.10	0.16	1.35	1.16	1.29	0.15	0.18	0.15
52.473	2941	2944	**Heptacosanal**	9.25	9.05	9.03	14.52	14.70	14.63	13.42	13.16	13.21	25.56	25.05	25.23	16.20	16.21	15.28
54.120	3039	3032	**Octacosanal**	1.72	2.13	2.12	3.04	2.70	2.93	4.06	3.46	3.46	12.01	11.34	11.82	2.04	2.28	3.07
Aldehydes unsaturated																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
7.403	1260	1254	**2-Decenal, (Z)-**	1.38	1.28	1.30	0.60	0.58	0.50	1.98	1.97	1.92	1.37	1.43	1.39	1.58	1.81	1.44
Unidentiffied																		
				FIBX1	FIBX2	FIBX3	ITBX1	ITBX2	ITBX3	LTBX1	LTBX2	LTBX3	LVBX1	LVBX2	LVBX3	NBX1	NBX2	NBX3
58.974	3345	N/A	**?**	3.53	2.94	1.25	1.01	1.01	1.01	1.54	1.54	1.54	2.82	1.29	1.29	1.44	1.44	1.44

5. Literature

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