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Article

# The Comparative Analysis of Agronomic Characters and Phenolic Composition of Blue Honeysuckle Berries (*Lonicera caerulea* L.) Cultivated in Central Yakutia

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**Abstract:** This study investigates the agronomic characteristics and phenolic composition of ten varieties of blue honeysuckle (*Lonicera caerulea* L.) cultivated in Central Yakutia. The research was prompted by the nutritional needs of the local population, particularly during the long winters when vitamin sources are scarce. The varieties included in the study were bred at various research institutes, with 'Goluboe Vereteno' serving as a standard due to its recommendation for the region. The study was conducted over the 2022-2023 vegetation seasons in Pokrovsk, Yakutia, focusing on identifying promising varieties that exhibit high yield, favorable taste, and significant polyphenolic content, which are essential for nutritional benefits. The findings highlight the potential of honeysuckle as a valuable crop in the region, contributing to local food security and agricultural diversity. The research underscores the growing interest in honeysuckle cultivation, which has gained traction in various countries, including Canada and Japan, where it is recognized for its health benefits and adaptability to different climates. Overall, this study contributes to the understanding of honeysuckle's agronomic potential in extreme northern conditions.

**Keywords:** selection; genetic resources; edible berries; north conditions; agriculture; berries

## 1. Introduction

In the conditions of the Extreme North, the use of wild and cultivated berry crops and the consumption of local products becomes a circumstance of a great importance in terms of nutritional wellbeing. Berry crops represent valuable sources of nutrients and biologically active substances for Yakutia [1,2]. People in Yakutia have a long winter without proper vitamin sources and early summer berries could make up the nutritional deficit. The most common berry crops in Yakutia are lingonberries, black and red currants, strawberries, blueberries, raspberries, and honeysuckle [3]. Recently, honeysuckle has begun to gain increasing popularity in this region [4].

In natural conditions, honeysuckle grows in the underwood of leafy and mixed forests, on rocky slopes, and near the banks of streams [5]. It was noted that the ecological area of sweet-fruited forms of honeysuckle is located near the valleys of mountain rivers [6]. Modern varieties of the crop are distinguished by large fruits of excellent taste, which are perfectly fit for processing. The chemical composition of honeysuckle berries provide their high nutritional value due to the rich content of macro and microelements [7], which allows us to classify honeysuckle as one of the most valuable

berry crops. Nowadays, an industrial production of honeysuckle are undeveloped and there is a need in more fresh berries and the products of their processing [8].

Honeysuckle characterized with the very early fruit ripening period, high content of vitamin C and phenolic compounds, which allows it to demonstrate antioxidative, immunomodulatory, antibacterial, antiviral, antifungal, antiallergic and other types of activity, and provide wide application in medicine, cosmetology, food industry, and agriculture [7,9,10]. Honeysuckle berries have up to 1800 mg of flavonoids per 100 g, and the great part of that (up to 1200 mg per 100 g) could be composed by anthocyanins, which forms the intense dark blue color of the fruits [11]. It has been also noted that 100 mg of honeysuckle berries accumulate up to 150 mg of vitamin C and 2500 mg of P-active compounds, which include 900-1400 mg of anthocyanins, 120-620 mg of catechins, 15-100 mg of phenolcarboxylic acids, and about 70 mg of flavonols and flavones [12].

The features of the variety and genetic characteristics of the parental forms are the main factors influence biochemical composition of honeysuckle berries [13,14]. Moreover, the accumulation of substances to a large extent depends on climatic conditions. The main environmental factors that affect the chemical composition of honeysuckle berries include the intensity of illumination, temperature and amount of precipitation [15]. It was previously shown that low temperature and high humidity have a positive effect on the content of ascorbic acid in honeysuckle berries [16]. The other work demonstrated dependence of the chemical composition of honeysuckle berries on the time of collection and the degree of ripening of the fruit [17].

Research on variety and selection of honeysuckle has been carried out all over the world. It is believed that honeysuckle cultivation first began in Russia [13]. However, active creation of industrial honeysuckle plantations in Russia began only in the 2010s. As of 2023, 134 honeysuckle varieties were approved for use and included in the State Register of Breeding Achievements of the Russian Federation (State Register of Breeding Achievements Approved for Use. Volume 1. Plant Varieties (as of 23.05.2023 <https://gossortrf.ru/publication/reestry.php>). Among the available honeysuckle varieties, the most widely used in industrial plantings are varieties 'Vostorg', 'Siny Utes', 'Yugana' bred by the Selection Center Bakcharskoye (Tomsk Region) and 'Berel' bred by the Lisavenko Siberian Research Institute of Horticulture of the Federal Research Center of Agriculture (Altai Region).

Since early 90s of the twentieth century a great interest in honeysuckle has begun to grow in Japan [18]. In the USA, honeysuckle has been known for quite a long time, but it has become widespread only in recent years [19,20]. An important role in the promotion of honeysuckle research was played by Canadian breeder Bob Bors (University of Saskatchewan), whose varieties 'Aurora', 'Tundra', 'Borealis', and the 'Indigo' group of varieties, obtained using leaf-like-calyx deepblue honeysuckle *Lonicera caerulea* var. *emphullocalyx* (Maxim.), have become widespread and recognizable in the world [21]. Canadian varieties are widely cultivated in North America, Europe, Asia, and even Australia [22,23]. In New Zealand, Japanese honeysuckle *Lonicera japonica* became a common weed after its introduction in 1872, and various measures and programs were developed for weed control. In Belarus, the gene pool of blue honeysuckle is represented by 30 varieties of Russian selection [24]. In the conditions of Ukraine, honeysuckle has been grown since the end of the 90s of the last century when the varieties 'Bogdana' and 'Fialka' were registered in the State Register. In 2001, the first varieties of local selection were created in the Donetsk Botanical Garden, namely 'Donchanka', 'Skifskaya' and 'Ukrainka' [25].

In 2020, Chinese breeders registered at the national level the first two honeysuckle varieties, namely 'Lanjingling' and 'Wulan', which was obtained from Russian varieties 'Berel' and 'Blue bird' (*L. caerulea* subsp. *kamtschatica*) [26]. Currently, China set the largest area for honeysuckle production, which is about 5,000 hectares, and it is aimed mainly for growing the varieties 'Lanjingling', 'Wulan', and 'Berel' [27].

The total area used for honeysuckle production in other countries is estimated at 5,500 ha [28]. About 1,000 ha of that land are used for honeysuckle production in Canada [28]. The honeysuckle planting area in Poland is pushing about 2,000–2,500 ha planted mainly with Polish varieties [10]. The total area of industrial honeysuckle gardens in Russia is about 700 hectares [29].

All modern honeysuckle varieties belong to the tetraploid polymorphic species *Lonicera caerulea* L., known as blue honeysuckle, which are naturally widespread in the boreal forest of northeastern Eurasia. Several endemic diploid species of the blue honeysuckle (*Caeruleae* Rehd.) are known for good taste, but they still have not been involved in the selection process. These species include *L. edulis* Turcz. ex Freyn (known as edible honeysuckle), *L. boczkamikowae* Plekh., and *L. iliensis* Pojark.

In Yakutia, there are three native honeysuckle species, namely edible honeysuckle (*L. edulis* Turcz.ex Freyn.), Altai honeysuckle (*L. altaica* Pall.) and Pallas's honeysuckle (*L. pallasi* Ledeb). Edible and Altai honeysuckle are common in all floristic regions of Yakutia, except for the Arctic and Kolyma regions. Pallas's honeysuckle was included in the list of higher vascular plants of Yakutia only in 2005, and it can be found in the southern and central regions, such as Verkhne-Lensky, Aldansky and Central Yakutsky region [5].

Despite the diversity of wild honeysuckle, traditionally the local population mostly have not been used it for storage. Interest in honeysuckle as a garden crop in Yakutia was formed in the 80s of the last century. Basically, honeysuckle bushes were transferred from natural conditions on farmlands and individual varieties of honeysuckle were brought from the Amur, Novosibirsk, and Khabarovsk region [5].

In 2004, Professor V.N. Sorokopudov brought 24 varieties of Altai honeysuckle for the Yakut Scientific Research Institute of Agriculture. During the study period, several samples did not take root in the conditions of Yakutia. The variety study was carried out by candidate A.A. Ivanov from 2004 to 2019. The results of the studies were published in a group of works [4,30,31].

Current study was performed at the Yakut Research Institute of Agriculture and assessed agronomic characters and the content of phenolic compounds and biosynthetically related quinic acid of ten varieties of blue honeysuckle berries (*L. caerulea* L.) cultivated in Central Yakutia in 2022-2023.

## 2. Materials and Methods

### 2.1. The Object of this Study

Ten varieties of blue honeysuckle were taken for the study (Table 1). Regional variety 'Goluboe Vereteno' was taken as a standard because it is recommended by the State Commission of Russia for the territory of Eastern Siberia and the Far East [32]. Five obtained varieties, namely 'Sinilga', 'Izyuminka', 'Stoykaya', 'Dlinnoplodnaya', and 'Lenita', were bred at the South Ural Research Institute of Fruit, Vegetable and Potato Growing. While four varieties, namely 'Goluboye vereteno', 'Berel', 'Krasnoyarochnka', and 'Sibiryachka', were bred at the Lisavenko Siberian Research Institute of Horticulture. The variety 'Ust-Aldanskaya' is a native domesticated form of honeysuckle obtained from Aldansky region of Yakutia.

**Table 1.** The characteristics of the blue honeysuckle samples used in the study.

Variety	Bush shape and productivity	Berries	Taste	Resistance
Goluboye vereteno (std.)	Rare and growthy; 1.5-2.5 kg/bush	large elongated, spindle-shaped moderately blue with flat base and a pointed tip; have a strong waxy coating. An average weight of one fruit is 0.97 g.; skin is dense, pulp is tender	Sweetish and sour taste with bitterness	High winter hardiness and resistance to diseases

Berel	upright, slightly branchy; 1.8–3.0 kg/bush	large berries, average weight of one fruit is about 1.3–1.6 g, oval, moderately pointed, almost black with a blue coating; berries do not crumble	Sweet and sour with a slight bitterness; medium density skin; the pulp is tender and juicy	early ripening
Sinilga	low-growing bush with average height of 0.5 to 1 meter (suitable for growing in vegetable gardens); average yield is 106 c/ha	very large, up to 2.2 grams; oval elongated shape, dark blue color, thin and waxy coating	Sweet with a fresh delicate sourness and very pleasant aroma	high winter hardiness, frost and drought resistance; weakly affected by diseases and pests
Izyuminka	weakly spreading, with average height of 1.2–1.3 m; average yield is 0.9–1.4 kg/bush, 27 c/ha	large with an average weight of one fruit about 1.1-1.6 g; oval elongated shape	Sweet, excellent taste.	resistant to low temperatures, diseases and pests
Krasnoyarochnka	growthy bush of 1.5–2.0 m in high; average yield is 1.9–3.5 kg/bush	medium size, average weight of one fruit is 0.72 g; bluish with a medium intensity waxy coating, oval with a rounded base and a pointed tip	Sweet and sour with a strong aroma	high winter hardiness and drought resistance
Sibiryachka	oval crown, medium height, moderately sprawling bush; the yield is up to 3.1 kg/bush	eardrop-shaped, dark purple, with whitish waxy coating; average weight of one fruit is about 1.0–1.4 g	Highlighted as one of the most delicious and sweet edible honeysuckles	high winter hardiness
Stoykaya	growthy, weakly spreading bush; average yield is 0.42–0.85 kg/m <sup>2</sup>	oval lumpy blue berries with dove-colored waxy coating; average weight of one fruit is 0.81 g;	Light bitterness in taste	winter hardiness and resistance to spot disease
Dlinnoplodnaya	low growing, strongly spreading bush with thin, curved purple shoots;	large fruits, average weight of one fruit is 1.5-2.5 g, elongated-flat, lumpy, blue with a bluish coating	Sweet and sour, dessert taste without bitterness	early ripening, high productivity, high winter hardiness

Lenita	1.5-2.4 kg/bush (up to 3 kg) grows, medium-spreading bush with thin, curved shoots; average productivity, 0.27 kg/m <sup>2</sup>	large fruits, average weight of one fruit is 1.0-1.5 g, elongated, blue with a bluish bloom	Sweet, excellent taste.	resistant to low temperatures, diseases and pests
Ust-Aldanskaya	oval crown, medium height; very low productivity	small fruits, average weight of one fruit is about 0.4 g, fruits are oval in form	Sour with bitterness	resistant to low temperatures, diseases and pests

## 2.2. Location and Conditions of the Study

The studies were conducted in the fruit and berry garden of the Yakut Research Institute of Agriculture, in the town of Pokrovsk, Yakutia (80 km south of Yakutsk) in 2022 and 2023.

The climatic conditions of Central Yakutia are sharply continental, temperature fluctuations reach 90–102°C. The sum of positive average annual air temperatures above 5°C is 1600–1800°. The sum of positive average annual air temperatures above 10°C is 1400–1500°. The overall average annual air temperature is –10.8°C. The absolute temperature minimum in Pokrovsk is –63.3°C. The sum of negative temperatures is 5000–6000°C. Snow falls in October and melts in mid- to late April. The snow cover depth is 25–38 cm [33]. Summer in Central Yakutia is short and dry. The average July temperature is 18.3°C, the maximum is 38.3°C. Frosts are possible in the summer months (from –3 to –9°C). The average frost-free period is 90–110 days [34].

The research field has permafrost, soddy forest, pale yellow soil. In mechanical composition, the soil is medium loamy, insufficiently provided with mobile forms of nutrients, namely phosphorus (15.2-21.0 mg/100 g of soil, according to Kirsanov) and exchangeable potassium – (20.6-25.3 mg/100 g of soil). The soil acidity varies from slightly to strongly alkaline (pH 7.4-8.3). The humus content in the 0-20 cm layer fluctuates from 2.5 to 3.0%.

## 2.3. Meteorological Conditions During the Study

In 2022, weather conditions during the vegetation period were characterized by late spring, hot and rainy July, and cool rainy autumn. In May, minimum temperatures dropped to –8.7°C at night, precipitation was 3.5 mm more than the long-term average (Table S1). It was hot in June and July. In the third decade of July, the temperature rose to +34.6°C. Precipitation in July was 40.1 mm more than normal, which had a favorable effect on the condition of plants. In the third decade of August, there were frosts of –1.6°C. Precipitation was within the norm. It was cool in September, minimum temperatures in the third decade dropped to –8.9°C at night.

In 2023, weather conditions during the vegetation period were characterized by cool May, rainy June and July, and cool autumn. In May, minimum temperatures dropped to –9.6°C at night, the level of precipitation was very little, namely 2.2 mm, less than the long-term average by 16.8 mm (Table S2). In June and July, the air temperature was at the level of long-term average values. In the first decade of July, the air temperature rose to 35.9°C. Precipitation in June exceeded the norm by 11.5 mm, and by 31.8 mm in July, which had a favorable effect on the condition of plants. It was cool in September, minimum temperatures in the third decade dropped to –12.6°C at night.

## 2.4. Methodology for the Assessment of Agronomic Characters

The used planting scheme was 2.5–1.5 m. The “Program and Methodology for Variety Study of Fruit, Berry and Nut Crops” was used for phenological observations, harvest records, and resistance to diseases and pests [35].

### 2.5. The Evaluation of Quinic Acid and Phenolic Compounds in Berries

Freshly picked honeysuckle berries were frozen to  $-70^{\circ}\text{C}$  and then lyophilized. A sample of dried berries in weight of 0.5 g was ground in a mortar, then 10 ml of 70% ethanol was added for extraction. The extraction was carried out for 12 hours at a temperature of  $20^{\circ}\text{C}$  and constant stirring. The obtained extracts were centrifuged at 4000 g for 10 min. Then 0.1 ml of the obtained centrifugate was transferred to separate vials and evaporated in a vacuum concentrator for 12 hours, after which it was used for analysis by gas chromatography-mass spectrometry (GC-MS). The remaining centrifugate was transferred to clean test tubes and evaporated in a vacuum concentrator for 24 hours. The dry residue in each test tube was dissolved in 2 ml of hot water. After cooling, the aqueous extract was extracted successively with 4 ml of diethyl ether and 4 ml of ethyl acetate. The upper organic layers were separated and transferred to clean tubes and evaporated in a vacuum concentrator for 12 h. The resulting dry residue was dissolved in 1 ml of 70% ethanol and then used for analysis by high-performance liquid chromatography (HPLC).

HPLC analysis was performed on a LicArt62 device (Labconcept, Russia) equipped with a reversed-phase column Agilent Eclips XDB-C18  $150 \times 2.1$  mm ( $80 \text{ \AA}$ ,  $3.5 \text{ \mu m}$ ). A mixture of water/acetonitrile/formic acid 95:5:0.1 was used as mobile phase "A", phase "B" was acetonitrile/water/formic acid 90:10:0.1. Gradient elution mode according the phase B was as follows: 0–5 min 5%, 5–8 min 5–15%, 8–10 min 15%, 10–15 min 15–40%, 15–20 min 40%, 20–25 min 40–100%, 25–30 min 100%, post time 10 min. The flow rate was 0.3 ml/min and a column temperature were  $30^{\circ}\text{C}$ . Injection volume was 5  $\mu\text{l}$ .

Detection of catechin and epicatechin was carried out on a fluorescence (FL) detector. The excitation wavelength was 280 nm, emission wavelength was 315 nm. Epigallocatechin gallate was recorded using a diode array detector (DAD) at wavelengths of 200–400 nm. Identification was carried out by comparing retention times and UV spectra of peaks on the chromatogram with the peaks of catechin standards from the collection of the Komarov Botanical Institute of the Russian Academy of Sciences.

For GC-MS analysis, 25  $\mu\text{l}$  of pyridine were added to the vials with dry centrifugate residue. To obtain volatile trimethylsilyl derivatives (TMS), derivatization was performed using 25  $\mu\text{l}$  of N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) for 15 min at  $100^{\circ}\text{C}$ . The analysis was performed on a Maestro chromatograph (название прибора или производителя, Russia) with an Agilent 5975C quadrupole mass spectrometer (USA), HP-5MS column,  $30 \text{ m} \times 0.25 \text{ mm}$ . A linear temperature gradient from  $70^{\circ}\text{C}$  to  $320^{\circ}\text{C}$  at a rate of  $4^{\circ}\text{C}/\text{min}$  with a gas flow (helium) of 1 ml/min was used for chromatography. Data were collected using Agilent ChemStation software (Фирма, Страна). Processing and interpretation of mass spectrometric data was performed using the AMDIS program (Фирма, Страна) and the NIST2011 standard library. Quantitative interpretation of chromatograms was performed using the internal method of standardization by hydrocarbon C23.

All measurements were performed with fresh samples in 3 biological and 3 analytical replicates.

### 2.6. Statistical Analysis

The results of the agronomic characters assessment were processed according to the field experiment method of Dospekhov [36] using the application software package SNEDEKOR and MS OFFICE EXCEL.

The results of HPLC-DMD-FL and GC-MS are presented as the arithmetic mean with standard error ( $M \pm SD$ ). Comparison of sample means was performed using one-way analysis of variance (ANOVA). The significance of differences between means was determined using the Newman-Keuls criterion for multiple comparisons at  $p < 0.05$ . The calculation was performed using the AnalystSoft package (Фирма, Страна), statistical analysis program StatPlus v.2007 (Фирма, Страна).

Hierarchical clustering by the Ward method was performed online in the MetaboAnalyst 6.0 program (<https://www.metaboanalyst.ca/>). Distances on the dendrogram were estimated using the Pearson correlation coefficient.

### 3. Results and Discussion

#### 3.1. Phenophases of Honeysuckle Plant Development

According to existing literature data, blue honeysuckle is considered as one of the most winter hardy berry plants [37]. It was noted that plants withstand the temperature below  $-40^{\circ}\text{C}$  and sometimes even  $-50^{\circ}\text{C}$  [38]. In the conditions of Yakutia, agricultural crops have additional risks caused by frequent returning frosts. However, buds, flowers and buttons of honeysuckle can withstand short-term frosts up to  $-6-8^{\circ}\text{C}$ . Winter damage of honeysuckle is extremely rare in Yakutia, and it occurs only in the years when a hard winter comes after a long, extremely warm autumn [39].

In Central Yakutia, honeysuckle is the crop with an earliest growing season [31]. The dates and duration of the main phenological phases of the honeysuckle development in 2022 and 2023 are represented in Table S3 and Table S4, respectively.

Among the samples evaluated in this study, the earliest growing season was registered for 'Krasnoyarochnka' variety, namely May 13 in 2022 and May 12 in 2023. The standard variety 'Goluboye vereteno' entered this phase a little later (May 17 in 2022 and May 14 and 2023). The 'Izyuminka' variety was the last sample started budding in 2022 (May 19) and the 'Stoykaya' variety was the last sample started budding in 2023 (May 19).

The local form 'Ust-Aldanskaya' had an early flowering stage and a low degree of fruiting compared to the other varieties. In both season, there was no secondary growth of shoots. The duration of the growing season for all the studied samples was 124-137 days in 2022 and 133-143 days in 2023. The 'Stoykaya' and 'Dlinnoplodnaya' varieties were the last to shed their leaves in both years of observation.

Over the two years of study, the honeysuckle varieties did not have winter damage. Spring observation also revealed no frost damage. Diseases or pests have not been observed. During the study, the most serious damage to the honeysuckle crop was caused by birds eating ripe fruits during the fruiting time.

#### 3.2. Agronomic Characters of the Studied Honeysuckles

The main agronomic characters of studied varieties are presented in Table 2.

In 2022, high berry yield was demonstrated by the 'Berel' variety (15.2 c/ha), which significantly exceeded the productivity of the regional standard variety 'Goluboe Vereteno' (9.3 c/ha). The third best result demonstrated the 'Krasnoyarochnka' variety (4.3 c/ha). The yield of the other samples varied from 0.6 to 3.1 c/ha. In 2023, the highest yields were also obtained by the 'Berel', 'Goluboe Vereteno', and 'Krasnoyarochnka' varieties, which were 11.4, 11.0, and 7.7 c/ha, respectively.

Considering size of the berries, varieties 'Goluboe Vereteno', 'Dlinnoplodnaya' and 'Krasnoyarochnka' had the biggest fruits. The highest result was demonstrated by 'Goluboe Vereteno' in 2023 with the mean weight of a berry as 1.0 g.

Excellent dessert taste is typical for the varieties 'Izyuminka', 'Goluboe Vereteno', 'Dlinnoplodnaya', and 'Lenita'. In current study, Izyuminka and Lenita varieties demonstrated the highest taste score, followed by 'Goluboe Vereteno', 'Dlinnoplodnaya', and 'Berel'. Moreover, the berries of the 'Berel' variety did not tend to fall off when ripening. In the contrary, fruit shedding is more typical for the standard variety Goluboe Vereteno (4 points).

The local variety 'Ust-Aldanskaya' demonstrated the lowest productivity characters among all the used samples.

**Table 2.** Agronomic characters of studied honeysuckle varieties in 2022 and 2023.

Variety name	Mean weight of one berry, g		Yield, c/ha		Taste, score		Berries shedding, score	
	2022	2023	2022	2023	2022	2023	2022	2023
Goluboye vereteno (std.)	0.90	1.0	9.3	11.0	4.9	4.9	3	4
Berel	0.69	0.71	15.2	11.4	4.6	4.4	0	0
Sinilga	0.65	0.59	1.3	1.1	3.5	3.7	1	1
Izyuminka	0.56	0.59	0.6	4.3	5.0	4.9	2	2
Krasnoyarochnka	0.85	0.89	4.3	7.7	3.9	4.1	2	2
Sibiryachka	0.57	0.55	1.9	1.8	3.8	3.7	3	3
Stoykaya	0.80	0.77	3.1	2.8	3.7	4.0	1	1
Dlinnoplodnaya	0.89	0.86	1.8	1.2	4.6	4.8	1	2
Lenita	0.62	0.65	2.3	4.5	5.0	4.8	2	2
Ust-Aldanskaya	0.44	0.43	-	-	2.5	2.5	0	0
LSD <sub>05</sub>	2022				1.54			
	2023				2.64			

LSD<sub>05</sub>, least significant difference post hoc test for 5% significance value.

The productivity of the studied varieties will be further discussed by comparison with the results of several existing research works used the same varieties in other regions. Therefore, it was previously shown that the 'Goluboe Vereteno' variety had an average yield of 36.7 c/ha in the 2016–2020 field tests in the Republic of Bashkortostan [40], 23.1 c/ha in the 2020–2022 tests in the Tambov Region [41], 49.2 c/ha in 2003–2013 in Moscow Region [42]. All these results exceed the yield of this variety in current research several-fold. The Berel honeysuckle was acknowledged as the “champion” in yield in many studies and showed the productivity of 40.0 c/ha in the 2016–2020 field tests in the Republic of Bashkortostan [40], 96.3 c/ha in the 1994–1998 field tests in the conditions of Altai [43], 76.6 c/ha in 1996–1998 [44] and 177 c/ha in 2003–2013 in Moscow region [42]. Despite the yield of the most productive honeysuckle varieties in Central Yakutia was significantly lower than the yield of the same varieties in other regions, further study required to identify the most promising varieties and increase their yields in the conditions of Yakutia.

### 3.3. Results of Biochemical Analysis

The combination of field tests with biochemical analysis of berry crops allows us to identify not only the varieties which are promising for breeding in a certain region but also to find genetic sources of valuable biologically active components.

Many studies have discussed the antioxidant properties of polyphenolic compounds found in honeysuckle [45,46]. The main classes of polyphenolic compounds in honeysuckle berries are flavonols (quercetin, rutin, quercitrin), flavanes (proanthocyanidins, catechins) and anthocyanins. [47,48]. Content of catechins in honeysuckle berries ranging from 2.4 to 6.2 mg/g depending on geographic origin [48]. It has been noted that the content of catechins and epicatechin in honeysuckle can be highly dependent on berry size and illumination conditions, while it does not show any dependence on temperature [49]. It is known that catechins exhibit a wide range of biological activity, including antioxidant, anti-viral and anti-cancer activities [50].

A chromatographic study of berries using the HPLC-DMD-FL method revealed the presence of three polyphenolic compounds of the catechin group, namely catechin, epicatechin, and epigallocatechin gallate. The GC-MS method detected quinic acid (a by-product of the shikimate biosynthetic pathway) and chlorogenic acid (a product of esterification of quinic and caffeic acids). The content of quinic acid and phenolic compounds in honeysuckle berries from the field tests of 2023 season is represented in Table 3.

**Table 3.** Content of quinic acid and phenolic compounds in berries of blue honeysuckle varieties in the conditions of Central Yakutia, 2023.

Variety name	The chemical content in dry mass of berries				
	quinic acid	mg/g chlorogenic acid	catechin	µg/g epigallocatechin gallate	epicatechin
Goluboye vereteno (std.)	24.1±1.7 <sup>b</sup>	1.2±0.1 <sup>a</sup>	25.9±1.9 <sup>a</sup>	13.7±1.6 <sup>d</sup>	3.8±0.2 <sup>b</sup>
Berel	29.9±2.1 <sup>b</sup>	0.8±0.1 <sup>b</sup>	49.2±1.9 <sup>d</sup>	12.3±1.5 <sup>d</sup>	3.2±0.1 <sup>b</sup>
Sinilga	24.9±2.0 <sup>b</sup>	1.0±0.1 <sup>a</sup>	4.3±0.4 <sup>e</sup>	3.3±0.5 <sup>c</sup>	13.1±0.6 <sup>c</sup>
Izyuminka	38.0±2.7 <sup>a</sup>	1.0±0.1 <sup>a</sup>	2.0±0.5 <sup>b</sup>	5.0±0.6 <sup>b</sup>	2.4±0.2 <sup>b</sup>
Krasnoyarochnka	25.3±1.8 <sup>b</sup>	0.7±0.1 <sup>b</sup>	9.3±0.9 <sup>c</sup>	3.6±0.4 <sup>c</sup>	5.7±0.2 <sup>b</sup>
Sibiryachka	32.4±2.3 <sup>a</sup>	1.3±0.2 <sup>a</sup>	20±2.5 <sup>a</sup>	9.0±0.8 <sup>a</sup>	1.2±0.1 <sup>a</sup>
Stoykaya	31.5±2.2 <sup>a,b</sup>	1.0±0.1 <sup>a</sup>	9.5±0.6 <sup>c</sup>	6.8±0.6 <sup>b</sup>	15.0±0.6 <sup>c</sup>
Dlinooplodnaya	37.1±3.1 <sup>a</sup>	1.4±0.2 <sup>a</sup>	16.4±1.8 <sup>f</sup>	3.8±0.3 <sup>c</sup>	5.6±0.3 <sup>b</sup>
Lenita	29.8±2.5 <sup>b</sup>	1.3±0.2 <sup>a</sup>	6.1±0.6 <sup>e</sup>	3.1±0.2 <sup>c</sup>	5.3±0.4 <sup>b</sup>
Ust-Aldanskaya	39.0±3.5 <sup>a</sup>	1.2±0.2 <sup>a</sup>	137.5±16.9 <sup>g</sup>	13.3±1.9 <sup>d</sup>	13.5±0.5 <sup>c</sup>

Results are presented as mean ± standard error (M±m). Mean values with the same letter superscripts are statistically indistinguishable (p<0.05).

It was shown that the highest content of quinic acid was detected in the berries of the 'Sibiryachka', 'Izyuminka', 'Stoikaya', and 'Dlinooplodnaya' varieties, and these values did not significantly differ the content of quinic acid in the local form of honeysuckle 'Ust-Aldanskaya' (Table 3).

The lowest content of chlorogenic acid was found in the berries of the 'Krasnoyarochnka' and 'Berel' varieties. The other studied samples had no significant difference in chlorogenic acid content between each other and compared with local variety 'Ust-Aldanskaya'.

'Ust-Aldanskaya' honeysuckle had the highest content of catechins which was up to 68 times higher than that in the berries of the other studied varieties. Moreover, the berries of 'Ust-Aldanskaya' variety had the highest content of phenolic compounds and biosynthetically related quinic acid. Among the other varieties, high content of quinic acid was observed for the 'Sibiryachka', 'Izyuminka', 'Stoikaya', and 'Dlinooplodnaya' honeysuckles.

The content of monosaccharides and organic acids in the berries estimated by GC-MS is represented in Table 4. The content of citric acid in berries was more than 10% of dry weight for the varieties 'Sibiryachka' and 'Ust-Aldanskaya'. More than 100 mg of free monosaccharides (glucose and fructose) in one gram of dry weigh was in the berries of 'Dlinooplodnaya', 'Sinilga', 'Goluboe Vereteno', 'Izyuminka', and 'Krasnoyarochnka'. The lowest sugar content was observed in the local honeysuckle form 'Ust-Aldanskaya' and 'Sibiryachka' variety. The ascorbic acid content varied between the tested varieties from 0.3 to 0.8 mg/g of dry weight.

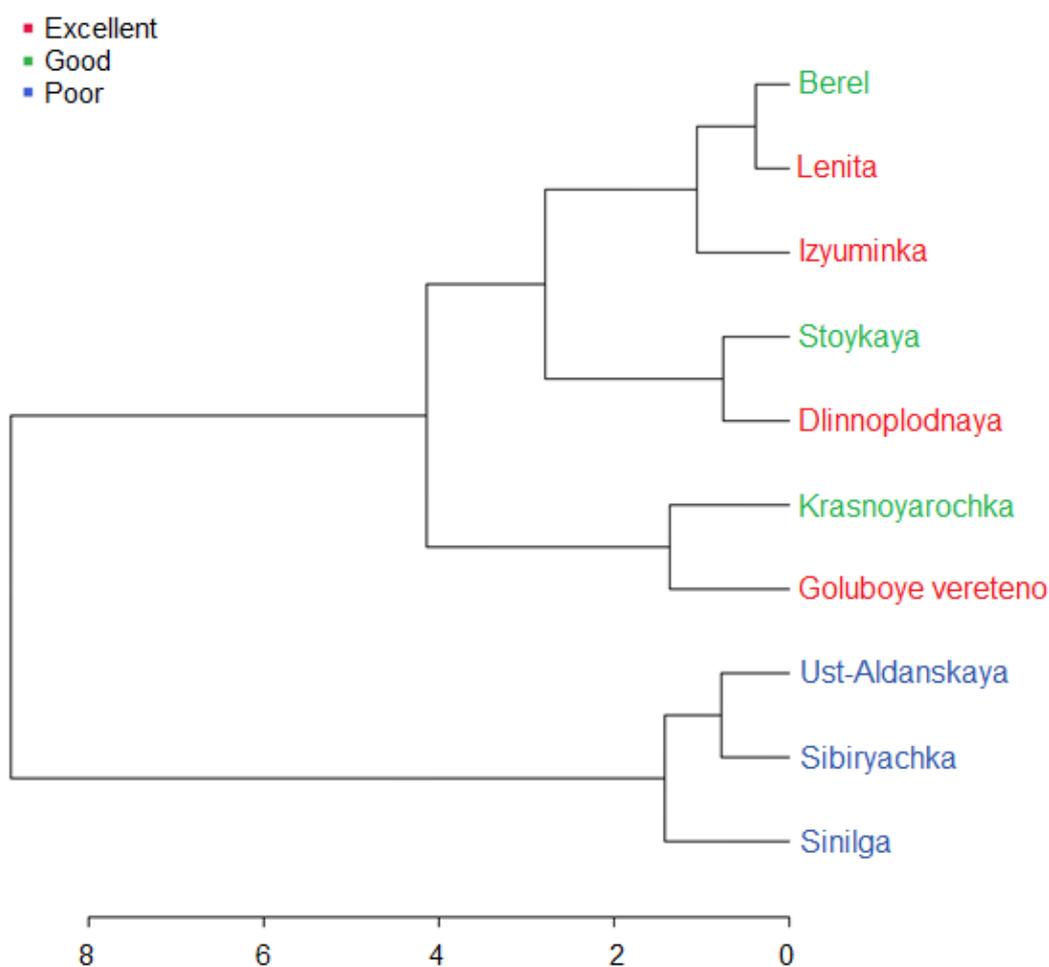
**Table 4.** Content of monosaccharides and organic acids in berries of blue honeysuckle varieties in the conditions of Central Yakutia, 2023.

Variety name	The chemical content in dry mass of berries, mg/g				
	citric acid	malic acid	fructose	glucose	ascorbic acid
Goluboye vereteno (std.)	96.4±10.6 <sup>a</sup>	23.1±2.4 <sup>a</sup>	94.5±2.5 <sup>a</sup>	54.5±2.2 <sup>a</sup>	0.4±0.0 <sup>a</sup>
Berel	86.7±5.2 <sup>a</sup>	52.5±3.2 <sup>b</sup>	70.3±3.4 <sup>b</sup>	20.8±2.0 <sup>b</sup>	0.8±0.1 <sup>b</sup>
Sinilga	97.9±8.9 <sup>a</sup>	41.3±2.5 <sup>b</sup>	64.9±9.1 <sup>b</sup>	86.8±5.0 <sup>c</sup>	0.5±0.0 <sup>a</sup>
Izyuminka	75.3±5.5 <sup>a</sup>	55.6±3.3 <sup>b</sup>	154.4±10.4 <sup>c</sup>	6.3±1.4 <sup>d</sup>	0.7±0.1 <sup>b</sup>
Krasnoyarochnka	85.9±5.2 <sup>a</sup>	39.5±2.4 <sup>b</sup>	102.4±10.0 <sup>a</sup>	100.1±9.3 <sup>c</sup>	0.6±0.1 <sup>a,b</sup>
Sibiryachka	129.3±14.4 <sup>b</sup>	26.2±2.6 <sup>a</sup>	22.6±1.4 <sup>d</sup>	20.3±2.0 <sup>b</sup>	0.3±0.0 <sup>a</sup>
Stoykaya	73.2±4.4 <sup>a</sup>	34.8±2.1 <sup>b</sup>	41±3.5 <sup>e</sup>	19.2±3.6 <sup>b</sup>	0.5±0.1 <sup>a</sup>
Dlinnoplodnaya	97.1±5.8 <sup>a</sup>	47.1±2.8 <sup>b</sup>	86.7±5.2 <sup>a</sup>	17±1.8 <sup>b</sup>	0.7±0.1 <sup>b</sup>
Lenita	95.4±5.7 <sup>a</sup>	44.9±2.7 <sup>b</sup>	69.9±4.2 <sup>b</sup>	13.7±1.0 <sup>b</sup>	0.4±0.0 <sup>a</sup>
Ust-Aldanskaya	177.1±12.6 <sup>b</sup>	23.9±2.4 <sup>a</sup>	29.6±2.4 <sup>d</sup>	12.3±1.7 <sup>b</sup>	0.4±0.0 <sup>a</sup>

Results are presented as mean ± standard error (M±m). Mean values with the same letter superscripts are statistically indistinguishable ( $p < 0.05$ ).

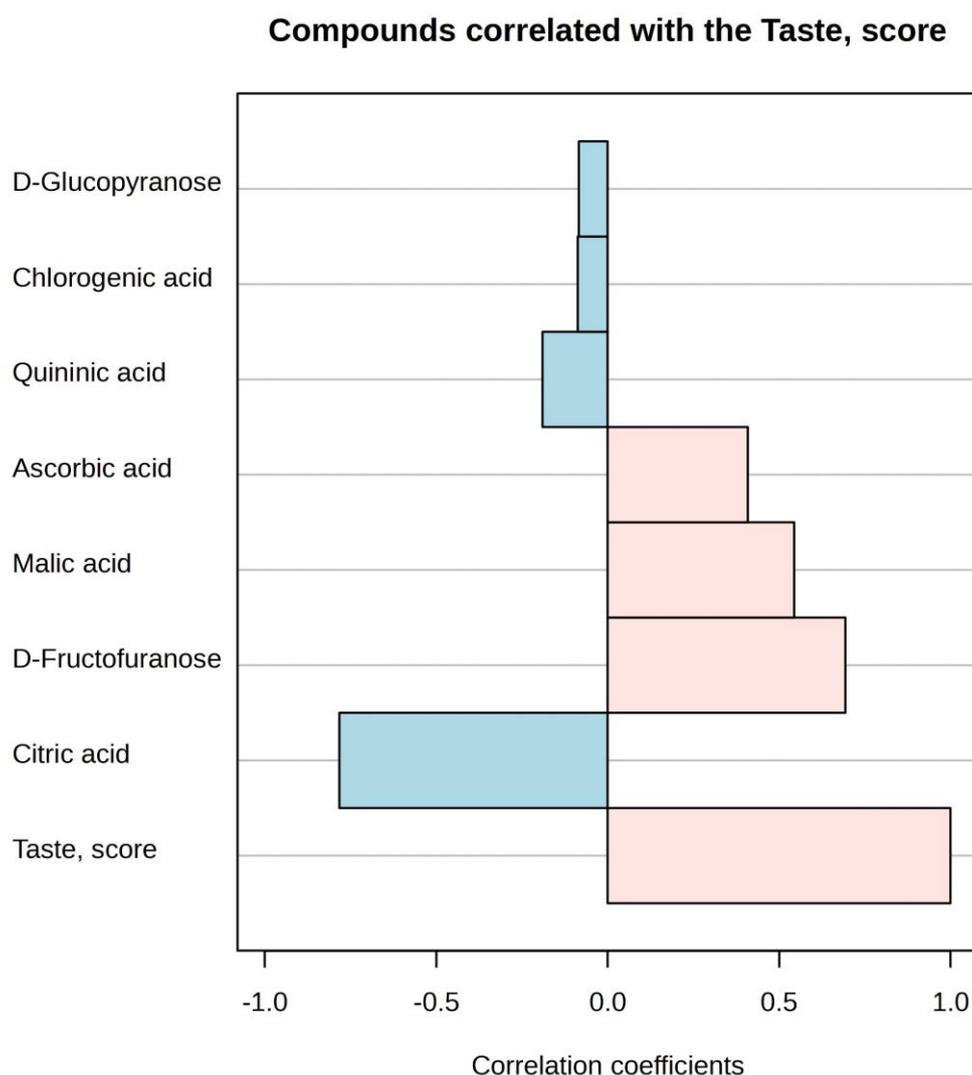
It is known that the sugar-acid index, which can be found as the ratio of carbohydrates to acids, the content of bitterness, and tannins are crucial parameters which determine the eating qualities of berries. We have used a cluster analysis to identify the relationship between the chemical composition and eating qualities of the berries (Figure 1).

The matrix was built using the data of phenolic compounds, quinic acid, monosaccharide, and organic acids content. The performed hierarchical clustering by the Ward method demonstrated that honeysuckle varieties can be grouped into two large clusters, according to the chemical composition and eating qualities of berries associated with that. It was shown that the first group included the varieties with excellent and good taste qualities (taste score more than 4.0 points) and the second group included the varieties with ordinary and poor taste qualities (taste score below 4.0 points).



**Figure 1.** Dendrograms of hierarchical clustering of chemical composition and taste qualities of blue honeysuckle berries using Ward's method. Distances between objects on the dendrogram were estimated using Pearson's correlation coefficient.

In order to identify the chemical components that make the highest contribution to the formation of the honeysuckle taste qualities, we have performed a correlation analysis (Figure 2). It was revealed that citric acid had the most pronounced negative contribution to the taste of the berries ( $r=-0.8$ ;  $p<0.01$ ), while the positive contribution was mostly made by fructose ( $r=0.7$ ;  $p<0.01$ ) and malic acid ( $r=0.7$ ;  $p=0.05$ ).



**Figure 2.** Correlation analysis of the relationships between the content of individual metabolites in the berry and its taste qualities (rated on a scale from 1 to 5).

The chemical structure, dissociation constant, and concentration of acid ions influence the perception of the sour taste of organic acids. It is known that an increase in the number of carboxyl groups at the same concentration and pH values of organic acids contributes to a decreased perception of acidity. Furthermore, low concentrations of organic acids have varying effects on the perception of sweetness in humans [51]. Thus, relatively low concentrations of ascorbic and malic acids in honeysuckle berries enhanced the taste qualities of the fruits, while high concentration of citric acid diminished this indicator. The improvement in taste quality with increasing concentrations of ascorbic and malic acids in honeysuckle berries may be related to the ability of low concentrations of organic acids to enhance the sweetness of certain sugars [52].

## 5. Conclusions

In this study we have tested 10 varieties of blue honeysuckle in the city of Pokrovsk (Yakutia) in 2022-2023 vegetation seasons. The results of this study revealed promising varieties for the region of Central Yakutia with the best yield, taste, and high content of polyphenolic compounds.

Among the tested varieties, 'Berel' demonstrated superior agronomic characters, high yield, large-fruitedness, and high winter hardiness in 2022 vegetation season. In 2023, according to the same

parameters, three varieties can be highlighted among the others, namely 'Goluboe Vereteno', 'Berel', and 'Krasnoyarochnka'. The best taste had the 'Izyuminka', 'Dlinnoplodnaya', and 'Lenita' varieties. Thus, these varieties can be recommended for further research in the conditions of Central Yakutia.

The results of biochemical analysis supplemented the superior yield characteristics of 'Goluboe Vereteno' and 'Berel' varieties with high content of phenolic compounds, primarily catechins. Therefore, these varieties can be considered as the most promising for the region.

At the same time, the 'Berel' variety has 1.9 times more catechins than the 'Goluboe Vereteno', which was used as a standard regional variety, and 2.5–24.6 times more catechins than the other studied honeysuckles except the local form of honeysuckle 'Ust-Aldanskaya'. The wild phenotype honeysuckle 'Ust-Aldanskaya' was characterized by the highest content of phenolic compounds among the studied varieties, while the yield and taste score of the local form were significantly lower compared to the other samples. Based on the current results we can conclude that the honeysuckle variety 'Ust-Aldanskaya' with a wild genotype represent an interest for further study as a source of phenolic compounds and as a source for breeding resistant honeysuckle varieties, but due to its low yield and taste qualities it is not suitable as a donor for obtaining a highly productive regionalized honeysuckles.

This work has presented a method for selecting honeysuckle varieties based on the taste qualities of the berries utilizing chemical analysis through hierarchical clustering. This method may facilitate a better and higher quality selection of new varieties during honeysuckle breeding in various climate conditions. Future works can include the identification the composition and quantity of flavonoids and anthocyanins in blue honeysuckle berries.

**Supplementary Materials:** The following supporting information can be downloaded at: Preprints.org, Table S1: Meteorological conditions of the vegetation period in 2022, Pokrovsk; Table S2: Meteorological conditions of the vegetation period in 2023, Pokrovsk; Table S3: The advent of phenological phases in the tested blue honeysuckle varieties in 2022, Pokrovsk; Table S4: The advent of phenological phases in the tested blue honeysuckle varieties in 2023, Pokrovsk.

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