The impact of cultivation systems on the nutritional and phytochemical content, and microbiological contamination of highbush blueberry

Ireneusz Ochmian, Magdalena Błaszak, Sabina Lachowicz & Renata Piwowarczyk

The aim of this study was to compare the nutritional and use value of berries grown in organic and conventional systems. The polyphenol content, fruit colour and firmness, and yeast, mould, and mycotoxin contents were assessed in blueberry fruit freshly harvested and stored for 8 weeks in controlled conditions (CA: CO₂-12%; O₂-1.5%, temperature 1.5 °C). The Shannon–Wiener diversity index was comparable in both systems and was lower for mould in organic fruit than in conventional fruit. Mycotoxins (deoxynivalenol, zearalenone) were found only in organic fruits. The optimal mineral content and pH of the soil allowed the cultivation of blueberry in accordance with organic standards. The storage of highbush blueberry fruit in CA cold storage for 8 weeks resulted in a slight deterioration in fruit quality and polyphenol content. The lower nutritional value of these fruits is compensated by the lack of pesticides and artificial fertilizers residues. The use of fungicides in conventional cultivation reduces the population of sensitive fungi and therefore reduces the contamination of fruits with mycotoxins.

Global production of the northern highbush blueberry (Vaccinium corymbosum L.) approaches nearly 655 thousand tons per year, and it has increased 20-fold within the last 20 years. Highbush blueberry production and processing occurs primarily in North America. The area under cultivation has increased substantially in China, Mexico, Poland and Spain. Growing demand for blueberry stems from the unique nature of the fruit, which has no commercial equivalent, its considerable nutritional value and high content of polyphenols, especially anthocyanins.

The demand for organic fruits is growing every year. This is due to the raising awareness of consumers who see the logical link between polluted agricultural produce, air and water, the increasing incidence of cancers, allergies and infertility. When optimum conditions are created for the growth and development of plants, organically grown fruit can attain very high quality, higher than that in conventional cultures, which is contrary to the conventional wisdom. Globally, approximately 5% of the total production area of blueberry is organically cultivated. Blueberry cultivation, also ecological, is not particularly demanding. First and foremost, an optimum soil (humus-rich and acidic) should be prepared for proper growth. Problems in the organic cultivation of the fruit arise especially during humid and warm years, with perfect conditions for mould fungi to colonize on the aboveground parts and the roots. Additionally, fungi as well as blueberry, prefer slightly acidic substrate/soil.

The weather is an important factor that has a direct impact on cultivated plants in recent years, it has become increasingly unpredictable. The increase in temperature is not the only issue. The considerable variations in precipitation levels during fruit ripening in individual years also pose problems. Blueberry cultures face particular
danger from *Glomerella acutata* (*anthracnose*), *Botrytis cinerea* (*gray mould*), *Godronia cassandrae* (*godronia canker*), *Botryosphaeria corticis* (*canker*), *Phytophthora* spp. (*root rot*), *Diaporthe* spp. (*twig blight and fruit rot*), and *Alternaria tenuissima*. In integrated organic cultivation, monitoring is the basis for the estimation of pest/phytopathogen infestation in the crop, as well as for the analysis of plant yield to determine potential causes for yield loss. The fungal infection and the growth of mould on the plant results in financial losses for the farming industry and poses a threat to consumer’s health. To become a source of mycotoxins, the mycelium does not have to be highly developed and thus visible to the consumer. The fungus grows into the substrate/product and penetrates the tissues and even the cells of the plant. The substrate mycelium is responsible for nurturing the entire mycelium, as well as the surface mycelium. Research shows that fruit, even when stored under refrigerated conditions, maybe a source of hazardous mycotoxins. Fungal infection of the blueberry fruit may be visible and/or organoleptically detected (visible fungal mycelium, changes in fruit and juice colour due to flavonoid decomposition). Worse still, fungal infection also may remain invisible, posing a health hazard. Mycotoxins have deleterious effects on human internal organs; they are invisible and not sensorically perceptible.

The aim of this study was to compare the nutritional and use value of berries grown in organic and conventional systems. A wide spectrum of the polyphenol fraction was selected as the comparison parameter, and the antioxidant activity and anti-diabetic effects of fresh highbush blueberry fruit were evaluated at harvest and after of controlled atmosphere (CA) storage. We also evaluated whether contact with fungicides reduced the mycotoxin content by restricting mould development.

### Results

#### Yeasts, moulds and mycotoxins.

The number of yeasts isolated from fruit varied widely (2.7–4.8 log_{10} CFU/g) (Table 1). Generally, it can be said that the count of yeasts isolated from organic fruit was lower than that isolated from conventionally grown fruit (both fresh and stored). On average, 0.5 log_{10} CFU/g less yeast inhabited fresh organic fruit than conventionally cultivated fruit (the latter fruits contained an average of 4.0 log_{10} CFU/g of yeast). After a period of cold storage, the number of yeast on fruit significantly decreased in most

| Blueberry fruit | Yeast log_{10}CFU/g | Mould log_{10}CFU/g | Fungi isolated from fruit (affiliation to genus, % of all isolated) | Shannon–Wiener index | Mycotoxins µg/kg |
|----------------|---------------------|---------------------|-----------------------------------------------------------------|---------------------|------------------|
| Organic cultivation | | | | | |
| Fresh | 1* | 3.1c | 1.8d | 63 Cladosporium | 0.457 ± 0.037 | Deoxynivalenol 2.59 ± 0.14c |
| | 2 | 3.9c | 3.7a | 84 Cladosporium | 0.284 ± 0.095 | – |
| Stored | 1 | 2.7f | 1.7d | 45 Cladosporium | 0.458 ± 0.024 | Deoxynivalenol 1.59 ± 0.13 Zearalenone 0.16 ± 0.01 |
| | 2 | 3.4de | 2.4cd | 76 Cladosporium | 0.237 ± 0.036 | – |
| Conventional cultivation | | | | | |
| Fresh | 1 | 3.2e | 3.3ab | 45 Aureobasidium | 0.476 ± 0.060 | – |
| | 2 | 4.8a | 3.9a | 45 Cladosporium | 0.462 ± 0.047 | – |
| Stored | 1 | 3.7d | 2.6cd | 88 Aureobasidium | 0.152 ± 0.075 | – |
| | 2 | 4.5b | 3.9d | 99 Aureobasidium | 0.024 ± 0.001 | – |

Table 1. Impact of the cultivation method and storage of blueberry fruit on the amount of yeast and mould and the composition and variety of fungi as well as the presence of mycotoxins in fruit. *1–2 field number. *Values followed by the same letter, within the same column, were not significantly different (p < 0.05) according to t-Tukey test. *Mean values ± SD.
moulds. The fruit weight losses in the tested cultivar were relatively low, with means in the range of 1.4–2%. The
ity. This may also have resulted in slightly higher weight loss after storage and a greater diversity of yeasts and
changes in fruit firmness can be caused by many factors, which may result in the deterioration of fruit qual-
number and diversity of fruit-colonizing fungi. However, these differences became insignificant after storage.
fruits from different fields (1 and 2) differed in firmness. Fruits from field 2 were less firm and had a greater
Changes in fruit firmness can be caused by many factors, which may result in the deterioration of fruit qual-
tional field No. 1, the number of yeasts significantly increased by 0.5 \log_{10} CFU/g with the storage time of these
The amount of mould grown on blueberry fruit, like the number of yeasts, was characterized by a wide
range (1.8–3.9 \log_{10} CFU/g) (Table 1). Even fruits taken for analysis from the same field at different points (1
or 2) differed significantly in their mould content, and these differences were statistically significant. The larg-
est difference was 1.9 \log_{10} CFU/g between fresh organic fruit harvested from fields No. 1 and No. 2. As with
yeast, more mould was found on the conventionally cultivated fruit (both freshly harvested and stored) than on
the organically cultivated fruit. After the cold storage period, the amount of mould on the fruit did not change
or significantly decrease (Table 1). The largest decrease in the amount of mould after storage was observed on
organic fruits harvested from field No. 2—by 1.3 \log_{10} CFU/g (Table 1). Fungi of the genus Cladosporium were
most frequently found in organically grown fruit (45–84% of all identified fungi). In conventionally grown fruit,
Aureobasidium was the most commonly found fungus (Table 1). Fungi belonging to eight different genera (Clad-
osporium, Fusarium, Penicillium, Acremonium, Alternaria, Aureobasidium, Bipolaris, and Mucor) were isolated
from organic fruits, and five genera of fungi were isolated from conventionally cultivated fruits (Cladosporium,
Penicillium, Alternaria, Aureobasidium, Eurotium). Although more types of fungi were found on the organic
fruit, the vast majority belonged to one dominant genus, i.e., Cladosporium (Table 1). This is why the mould
from organic fruit had a low value for the biodiversity index (in the case of organic fruits from field No. 2, the
Shannon–Wiener index = 0.284 ± 0.095). Conventionally cultivated fruits were also inhabited by large quantities
of fungi belonging to the genus Aureobasidium; therefore, a higher Shannon–Wiener diversity index value was
obtained. After storage, the moulds found on the fruit belonged to only 2–3 genera (in the case of conventionally
cultivated fruit, 88–99% of isolates belonged to Aureobasidium). Fusarium was not present in the conventionally
cultivated fruit, and this genus could not be identified in fresh or stored fruit, or in stems or leaves. Interestingly,
Fusarium fungi were isolated from fruits on one side of the organic field. The total count of moulds on these
fruits did not decrease even after 8 weeks of cold storage, and the share of Fusarium in the total number of fungi
colonizing the fruit increased from 15 to 27%. The presence of deoxynivalenol, a Fusarium mycotoxin, was
also identified in these samples (mean 2.59 ± 0.14 and 1.59 ± 0.13 µg/kg in fresh and stored fruit). Zearalenone,
another toxin, was detected only after the storage period and was not found in fresh fruit. No ochratoxin A,
toxin T2 HT2, aatitoxin (B1, B2, G1, G2), or patulin were identified in the tested samples of fresh and stored fruit.
Fungi of the genus Aureobasidium commonly colonized the fruit, and their share of the total fungi increased
considerably in the stored fruit originating from both organic and conventional cultures. In fresh organically
cultivated fruit, they constituted less than 1% of all isolated strains, whereas in the same fruit after 8 weeks of stor-
age, they increased to over 20% of the total fungi. Side shoots with leaves and main shoots of organically grown
blueberries were inhabited by a more diverse group of moulds than those of conventionally grown blueberries.
Moulds of the genus Fusarium sp., Triposporium sp. Pestalotiopsis maculans, Stemphylium sp., were only detected
in plant material from the organic farm (Table 2).

| Part of the plant | Dominant fungal species | Conventional cultivation |
|------------------|-------------------------|--------------------------|
| Side shoots with leaves | Alternaria alternata Botrytis cinerea Fusarium sp. Cladosporium herbarium | Alternaria alternata Botrytis cinerea |
| Main shoots | Phomopsis vaccinii Phytophthora sp. Rhizoctonia solani Triposporium sp. Pestalotiopsis maculans Stemphylium sp. | Phomopsis vaccinii Phytophthora sp. Rhizoctonia solani Colletotrichum geosporioides Botrytis cinerea |

Table 2. Differences in the mould composition from the leaves and shoots of the highbush blueberry, depending on the method of cultivation.

Firmness, color, and weight loss of fruit. The influence of the cultivation method and storage time
on the physical parameters of the fruit was also examined (Tables 3 and 4). It is difficult to determine whether
the confirmed presence of moulds, yeasts, and yeast-like fungi in the examined fruit affected their tenderness
or colour. After 8 weeks of CA cold storage, firmness decreased by less than 14% (Fig. 1). Fruit size has a deci-
sive impact on fruit quality. Smaller fruits harvested on a conventional plantation were firmer and less prone
to mechanical damage (Table 3), and had higher amounts of bioactive compounds. These fruits remained firm
after 8 weeks of storage (Table 4). Despite the variation in the levels of specific pathogens on fruits from differ-
ent fields, their firmness was similar, as was their resistance to mechanical damage. However, organically-grown
fruits from different fields (1 and 2) differed in firmness. Fruits from field 2 were less firm and had a greater
number and diversity of fruit-colonizing fungi. However, these differences became insignificant after storage.
Changes in fruit firmness can be caused by many factors, which may result in the deterioration of fruit qual-
ity. This may also have resulted in slightly higher weight loss after storage and a greater diversity of yeasts and
moulds. The fruit weight losses in the tested cultivar were relatively low, with means in the range of 1.4–2%. The
greatest weight loss was found in fruits harvested from the first plot of the organic plantation (2.4%). A higher biodiversity of fungi and their metabolites (mycotoxins, deoxynivalenol and zearalanone) were found on these fruits.

Independent of the cultivation method, the fresh fruits had similar $L^*a^*b^*$ colour parameters. Though similar in colour, the fruits harvested from conventional plantation had considerably higher contents of polyphenol compounds, including anthocyanins. The difference was significant, with 313.71 mg/100 g of anthocyanins

| Parameters                          | Fields number | Organic cultivation | Conventional cultivation | Mean |
|-------------------------------------|---------------|---------------------|--------------------------|------|
| Weight of 100 berries (g)           | 1             | 365a*               | 227b                     | 296A |
|                                     | 2             | 388a                | 196b                     | 292A |
|                                     | Mean          | 377A                | 212B                     |      |
| Fruit firmness (G/mm)               | 1             | 189b                | 217a                     | 203A |
|                                     | 2             | 172c                | 230a                     | 201A |
|                                     | Mean          | 180B                | 224A                     |      |
| Puncture resistance (G/mm)          | 1             | 137a                | 125a                     | 131A |
|                                     | 2             | 126a                | 131a                     | 128A |
|                                     | Mean          | 132A                | 128A                     |      |

Table 3. Quality and colour of fresh blueberry fruit, depending on the cultivation method. *Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to t-Tukey test; lower-case letters indicate interaction and capital letters the main factors.

| Parameters                          | Fields number | Organic cultivation | Conventional cultivation | Mean |
|-------------------------------------|---------------|---------------------|--------------------------|------|
| Fruit firmness (G/mm)               | 1             | 156ba               | 192a                     | 174A |
|                                     | 2             | 141b                | 205a                     | 173A |
|                                     | Mean          | 149B                | 199A                     |      |
| Puncture resistance (G/mm)          | 1             | 122a                | 119a                     | 121A |
|                                     | 2             | 118a                | 127a                     | 123A |
|                                     | Mean          | 120A                | 123A                     |      |
| Weight losses (%)                   | 1             | 2.4c                | 1.3a                     | 1.8A |
|                                     | 2             | 1.7b                | 1.5ab                    | 1.6A |
|                                     | Mean          | 2.0A                | 1.4B                     |      |

Table 4. Differences in the quality and colour of highbush blueberry fruit after CA cold storage, depending on the cultivation method. *For explanation, see Table 3.
determined in the organic fruit and 448.65 mg/100 g determined in the conventional fruit. Anthocyanins are largely responsible for fruit colour. The fruits, especially the organic fruits, darkened during storage (Fig. 1).

A change in the $L^*$ colour parameter indicates that the blueberry fruit is darkening. Irrespective of the cultivation method, the fruits changed from reddish to blue shades, as indicated by considerable changes in the $a^*$ and $b^*$ colour parameters. These changes were more pronounced in organic fruit than in conventional fruit. However, the changes in the content of anthocyanins were more pronounced in the conventional fruit, where the anthocyanin content decreased by 44% compared to that in fresh fruit.

The study demonstrated that storage had little effect on the reduction of the antioxidative effects of the blueberry fruits as determined by ABTS·+, DPPH and FRAP (Table 5). A decrease in inhibitory activities was observed after the storage period, but mainly in the organic fruits ($\alpha$-amylase IC$_{50}$ 27.33, $\alpha$-glucosidase IC$_{50}$ 19.68 mg/mL). In conventional fruits, the level of inhibitory activities was higher, at 20.63 and 15.07 mg/mL, respectively. The changes in antioxidative effects and inhibitory activities may have resulted from the decrease in polyphenol content as well as from the decrease in L-ascorbic acid in fruit after the storage period. Contrary to

Figure 1. Changes in physical (a) and colour parameters (b) of highbush blueberry fruit after CA cold storage.

|                      | Organic cultivation | Conventional cultivation |
|----------------------|---------------------|--------------------------|
|                      | Fresh fruits        | Stored fruits            | Fresh fruits        | Stored fruits            |
| ABTS·+ (µmol/g)      | 19.33b              | 17.06c                   | 22.57a              | 21.88a                   |
| DPPH (µmol/g)        | 17.42b              | 15.69b                   | 20.44a              | 19.83a                   |
| FRAP (µmol/g)        | 7.61b               | 7.94b                    | 9.11a               | 9.35a                    |
| $\alpha$-amylase IC$_{50}$ (mg/mL) | 24.48b            | 27.33a                   | 22.28bc             | 20.63c                   |
| $\alpha$-glucosidase IC$_{50}$ (mg/mL) | 18.47b             | 19.68a                   | 15.53c              | 15.07c                   |
| L-ascorbic acid (mg/100 g) | 128c              | 115d                     | 168a                | 142b                     |
| NO$_3$ (mg/1000 g)   | 43.1b               | 48.9a                    | 35.5c               | 40.7b                    |
| NO$_2$ (mg/1000 g)   | 0.17c               | 0.19bc                   | 0.21b               | 0.24a                    |

Table 5. Changes in health promoting capacities of highbush blueberry fruit after CA cold storage. *Values followed by the same letter, within the same line, were not significantly different (p < 0.05) according to t-Tukey test.
popular opinion, the fruit from the conventional plantations had higher nutritional value, i.e., higher antioxidant activity, anti-diabetic effects and polyphenol content (Tables 5 and 6).

**Polyphenol compounds and health promoting capacities.** Fruits harvested from the test fields of the organic as well as the conventional plantation (fields 1 and 2) were characterized by similar polyphenol compound contents (Table 6). The profile of polyphenols in blueberry fruits included six phenolic acids, including hydroxycinnamic acid, and their derivatives (three compounds), flavonols and their derivatives (15 compounds), flavan-3-ols (six compounds), and anthocyanins (nine compounds) (“Supplementary Information”). The polyphenol profiles of the organic and conventional fruit were identical. However, the total polyphenols were 22% higher in fruits harvested from conventionally grown bushes. During CA cold storage, the amount of polyphenols was reduced. Regardless of the cultivation method, anthocyanins comprised the largest group of the identified compounds. Anthocyanins also showed considerable changes in amount after storage. In fresh organic

### Table 6. Polyphenol content in fresh and stored blueberry fruit cultivated organically and conventionally (mg/100 g). For explanation, see Table 5.
fruit, the anthocyanin content was 313.71 mg/100 g, and in conventional fruit, it was 448.65 mg. After 8 weeks of CA cold storage, the content of anthocyanins decreased by 31% and 38%, respectively. The amount of delphinidin-3-O-glucoside decreased in both organic and conventional fruits. Moreover, considerable quantitative and percentage changes occurred in the contents of petunidin-3-O-glucoside and malvidin 3-galactoside. Blueberry fruits were also rich in phenolic acids, especially chlorogenic acid (181.32–232.41 mg/100 g), which is classified as a subclass of hydroxycinnamic acids. Together with anthocyanins, phenolic acids made up 74–89% of all the identified polyphenols. The phenolic acid content was similar in both types of fresh fruit (Table 6). However, the amount of the following phenolic acids decreased in organic fruit after storage: neochlorogenic, chlorogenic, and cryptochlorogenic acids. On the other hand, the content of cryptochlorogenic acid increased in conventional fruit, from 1.17 at harvest to 2.62 mg/100 g after storage. Similar trends were observed for flavan-3-ols. After storage, the procyanidin content (Tr. 3.96) increased considerably in organic and conventional fruits, from 11.16 to 57.80 mg/100 g in organic fruit and from 13.70 to 26.07 mg/100 g in conventional fruit. Moreover, the catechin content was found to increase considerably in conventional fruit after storage.

Conventionally grown highbush blueberry fruits were characterized by higher antioxidant activity than organically grown fruits as determined using ABTS+, DPPH and FRAP tests. However, this may have resulted from the fact that the organic fruits were much larger (mean weight of 100 fruits 377 g) than the conventional fruits (212 g). In the FRAP assay, the ability of blueberry extracts to reduce Fe3+ to Fe2+ ranged from 7.61 to 9.35 μmol/g. The free radical scavenging activity determined by DPPH varied from 15.69 to 20.44, and the values determined by ABTS+ ranged from 17.06 to 22.57 μmol/g (Table 5). Storing conventionally grown fruit in CA cold storage did not have a great impact on the polyphenol and L-ascorbic acid content, antioxidant activity or anti-diabetic effects of the fruit. A slight decrease in the α-amylase and α-glucosidase inhibitory effects was found in the organic fruits. L-ascorbic acid and polyphenols (especially anthocyanins) are characterized by high antioxidant activity. The conventional fruit was characterized by a higher content of these compounds, potentially resulting in higher antioxidant activity. Polyphenols, especially anthocyanins, can affect antioxidant activity. Anthocyanins are mostly found in the skin of berries. The high amount of phenolic compounds can have a positive effect on health. Berries from organic plantations were larger than those grown on conventional farms. To grow blueberries organically, the right conditions must be met. The most important factors are a suitable humus content in the soil, low soil pH and the location of the plantation. Bushes on the organic plantation had these optimal conditions—the substrate had low pH and high humus content. This resulted in the production of large fruit. The bushes on the conventional plantation grew in sandy soil, which had to be supplied with water and fertilizer.

It should also be noted that the fruits contained very low levels of harmful nitrates and nitrites. In fresh fruit, the organically grown fruit had the highest level of nitrates (43.1 mg/1000 g), while conventional fruits had higher levels of nitrites (0.21 mg/1000 g). There was a slight increase in these substances during storage, but their levels remained significantly below the standards.

Discussion

Yeasts, moulds and mycotoxins. Typically, saprophytic genera (Penicillium, Alternaria, Acremonium, Mucor, Aureobasidium), and phytopathogenic fungi (Bipolaris sp., Botrytis cinera, Phomopsis vaccini, Phytophthora sp., Rhizoctonia solani, Cladosporium sp., Fusarium sp.) were isolated from fruits, side shoots with leaves, and main shoots. These fungi are commonly found on fruit, in soil and on agricultural products and plant materials regardless of the climatic zone. Fresh fruits, including blueberries, demonstrate considerable tendency to infection by fungi that occurs during cultivation, harvest, transport, sale, and preparation for consumption. Visual and compositional fruit quality, as well as nutritive value changes, normally take place during storage.

Fruits have many simple sugars and organic acids; therefore, they are a good medium for yeast and mould. Colonization of the blueberry fruit by fungi depends on microhabitat conditions, numerous abiotic and biotic factors, and their interactions. Ecological agriculture systems that do not include the use of pesticides and synthetic fertilizers are thought to promote the biodiversity of a given habitat. The dominant type of fungi in organic cultivation was Cladosporium sp. (63–84% of all isolated fungi), and the fruit in conventional cultivation was most frequently inhabited by fungi from two genera: Cladosporium sp. (37–45%) and Aureobasidium sp. (42–45%). Other fungi belonging to other genera constituted only over a dozen percent of all isolated individuals. Because there was one clearly dominant genus—Cladosporium sp.—the biodiversity of organic fruit fungi (measured by the Shannon–Wiener diversity index) was comparable to or even lower than that of conventional fruit. The Shannon diversity index is high when there is a significant number of different species with individuals of similar abundance. Therefore, our observation does not support the idea that the ecological system of plant cultivation supports biodiversity. However, fungi belonging to 8 different genera were isolated from organic fruit, while only five genera were isolated from conventional fruit. Similarly, a richer set of taxa characterized the fungi isolated from organic plants (side shoots with leaves and main shoots). In addition, it should be noted that certain fungi (Bipolaris, Acremonium, Mucor, Fusarium) were found only on organic fruit. Considering the above, along with the observations and results of other authors, it can be assumed that the cultivation system (organic or conventional) had a significant impact on fungi. The cultivation system significantly affected the number of yeasts and moulds as well as the taxonomic composition of fungi. However, this impact could not always be considered as promoting biodiversity, as evidenced by the Shannon–Wiener diversity index.

The strong competition among these fungi does not allow the excessive development of a selected group of phytopathogens. However, the presence of mycotoxins has been detected in ‘Brigitta Blue’ fruit grown organically. This was probably related to the presence of fungi of the genus Fusarium on these fruits. These fungi were only present on fruits from the organic farm; the fungicides used in conventionally grown fruit could have
Fruit firmness, colour and weight loss. Blueberry fruit contaminated with fungi may lose some nutritional value and firmness due to the enzymatic activity of fungi. Mould fungi penetrate deep into plant tissues through microinjuries and secrete enzymes, such as pectinases, lipases, and cutinases, to damage the epidermis of the berries. Loss of firmness may also occur due to fruit decomposition and metabolite secretion. Due to blueberry water loss sensitivity, long storage can develop wilting and softening symptoms which has a negative effect on firmness. Postharvest water loss and turgor pressure drop are more dramatic for the harvested crops compared with water stress occurring in the field, because of the cell's inability to replace the water content from the vascular system. The firmness of fruits determines their resistance to mechanical damage. Other studies have shown that smaller fruits had higher firmness. The higher firmness of conventional fruits and their puncture is the result of their smaller weight, as in other studies. This contradicts the common opinion that organic fruit is of inferior quality. Despite the similar content of mineral components in the soil on both plantations, supplemented to an optimal level with fertilizers, irrigation or frost protection, organic fruit was much larger. The shrubs that grew on the ecological plantation were also much higher. The influence of the weather on the growth of shrubs can be excluded, because the plantations were at the same altitude. This is confirmed by numerous opinions that highbush blueberry shrubs should be planted in soil with a high level of organic matter and low pH value. Optimal growing conditions may also affect the higher resistance of the plants to pathogens, which allows them to be grown without plant protection products.

Fruit firmness is of decisive importance in assessing, among other things, fruit resistance to mechanical damage. It is commonly assumed that such a phenomenon is less intensive in fruits that are well supplied with calcium. Calcium is mainly transported to the leaves, and even a high Ca content in the soil does not guarantee that the fruit firmness will be high. In this study, we observed a decrease in fruit firmness after storage. Larger changes in both the firmness and the puncture resistance of the fruit were observed in fruits from organic farming. The mean firmness change after storage was 13.9% and was similar to the value of 15% reported by Chiabrando and Giacalone. Fruit weight loss in ‘Brigitta Blue’ was relatively low, from 1.3 to 2%, lower than the 3–5% previously observed after a 45-day storage period at 0 °C. In comparison, weight loss was as high as 15% after 9 weeks of storage under traditional storage conditions. Independent of the cultivation method, the fruits showed similar weight loss during storage. Weight loss in blueberries is mainly due to water loss caused by transpiration and respiration processes, which depend on the gradient of water vapor pressure between the fruits and the surrounding air. In addition to firmness, total acidity, soluble sugar content, and health-promoting elements, consumers highly rate colour of fresh blueberry. The colour of berries is caused by the presence of various bioactive compounds. Orange, red, purple and blue colours are related to the presence of pigments such as anthocyanins, carotenoids and betalains. Changes in the colour of the fruit indicate ripening. A change in the L* parameter, indicating blueberry fruit darkening, was also observed by Chiabrando and Giacalone. Fruits of the Brigitta blue cultivar were also darker after storage, as indicated by a decrease in L* colour parameter. Organic fruits at the time of harvest were darker than conventional fruits despite being harvested at the same time. It was also found that the change in the colour parameter of organic fruits was greater than that in conventional fruits. The nature of these changes was similar to that in the fruits of the Sunrice cultivar.
Ścibisz et al. demonstrated a significant correlation between anthocyanin content and $L^*$ value in experiments with highbush blueberry fruit. However, the research showed that the fruit, despite having similar colours, had a varied contents of anthocyanins. Those harvested from conventional plantations had a much higher content of polyphenolic compounds, including anthocyanins.

**Polyphenol compounds and health promoting capacities.** Due to the high content of polyphenolic compounds and high antioxidant activity, blueberries are considered to be functional foods. Polyphenols, especially anthocyanins, are mainly found in the skin of blueberries. Smaller fruits have a higher polyphenol content. This is due to the larger skin area of small fruit compared to that in the same amount of large fruit by weight, e.g., 100 g. Moreover, blueberries are characterized by low levels of ingredients that are considered to be toxic. Nitrates are not a major health risk to consumers, but nitrites are produced during the partial reduction of NO3. This process may intensify during transport or storage in conditions with low oxygen content. The presence of these compounds results from fertilization but also from the natural nitrogen cycle. Additionally, cultivation on peat soils, which are rich in organic matter, leads to higher accumulation of nitrates in plants. In sandy soils, nitrate ions are easily leached out. This may explain why the content of harmful compounds in the organic fruit was higher than that in the conventionally grown fruit.

However, the contents of harmful nitrates (max. 48.9) and nitrites (max. 0.24 mg/1000 g) in the tested fruits were low. In accordance with applicable regulations, these fruits can be considered safe for the consumer. Nitrate content limits are set only for green leafy vegetables in EU legislation. Fresh lettuce may contain up to 5000 mg/1000 g nitrate, and processed foods for feeding infants and young children should not exceed 200 mg/1000 g nitrate. In contrast, nitrite levels should not exceed 0.07 mg per kg body weight per day.

In previous studies, the antioxidant activity values measured using ABTS+ ranged from 811 to 3829 μmol/100 g. Differences in antioxidant activity result from the differences among cultivars and among production locations. The levels of antioxidant activity determined by the DPPH and FRAP methods in Bluecrop fruit were 1244 and 700 μmol/100 g, respectively. In both fresh and stored fruits in this study, the activity determined by DPPH was at a higher level than that found in a previous study (15.69–20.44 μmol/g), and the activity determined by FRAP was at a similar level (7.61–9.35 μmol/g). However, it was found that conventional fruit had a higher polyphenol content and a higher FRAP value than organic fruit. A linear relationship was observed between the total phenolics and FRAP values for blueberries. A similar relationship was also observed in 'Brigitta Blue' in this study.

The content of polymeric procyanidins is strongly correlated with the inhibitory activity towards α-amylase and α-glucosidase. Proanthocyanidin-rich blueberry cultivar extracts had the lowest IC50 value (25.0 mg/mL), suggesting a high α-glucosidase enzyme inhibitory potential. The tested fruit, both fresh and after storage, regardless of the method of cultivation, had a higher ability to inhibit α-glucosidase enzymes (IC50 15.07–19.86 mg/mL) in a previous study. The high content of polyphenols in the fruit could have an impact on the strong inhibition of α-amylase and α-glucosidase. The tested fruits were rich in anthocyanins, which inhibit α-glucosidase activity and can reduce blood glucose levels after starch-rich meals. Polyphenols are resynthesized products that protect against ultraviolet radiation and pathogens. Anthocyanin compounds are mainly concentrated in the skin of berries. Approximately half as many polyphenols and up to ten times fewer anthocyanins were determined in 'Bluecrop' than in this study. The tests were carried out using a different method, but the very small amounts of anthocyanins call into question the effectiveness of this method for determining these compounds. According to the literature, the anthocyanin content in different blueberry cultivars calculated as equivalents is between 22 and 497 mg/100 g. In contrast, in blueberry fruit, the amount of anthocyanins at full maturity determined by HPLC ranged from 245 to 684 mg/100 g. According to Reque et al., storing blueberries for six months at −18°C resulted in an average anthocyanin degradation of 59%. On the one hand, the anthocyanins contained in plants protect plants from infections because the anthocyanins show antifungal activity. However, some microorganisms are able to biodegrade anthocyanins. Microorganisms adapt quickly to the given environmental conditions, and their enzymatic potential, genetic variability and reproductive rate make them capable of neutralizing or even biodegrading many compounds that are toxic to living organisms and using them to obtain macromolecules and/or energy. Given this information, it seems clear that the microbiological decomposition of polyphenols, tannins and even the cuticle of the epidermis is possible. Connor et al. concluded that fruit gathered prior to full ripening can be stored for seven weeks without losses of antioxidants such as flavonols and anthocyanins. However, the polyphenol content of blueberry fruit
depends on the phase of ripeness. In some cultivars, the content of polyphenols increases during ripening, while in others, it decreases. A decrease in the content of polyphenols can also occur during storage68,69.

Conclusion
Optimal environmental and soil conditions enabled the organic production of blueberry bushes under certified ecological standards without the use of pesticides and fertilizers. The berries from the organic plantation were larger than the conventionally grown berries by approximately 80% but contained fewer bioactive compounds, namely, polyphenols and L-ascorbic acid. The organic growth conditions also resulted in lower antioxidant activity and less α-amylase and α-glucosidase inhibition.

The storage of highbush blueberry fruits in CA cold storage influenced the decrease in polyphenolic compound content; however, it had no significant influence on the decrease in antioxidant activity or on the effectiveness of α-amylase and α-glucosidase inhibitors.

Regardless of the method of cultivation, after storage, the highbush blueberries became darker, and their firmness decreased.

Fungi that were present on both organic and conventional fruit belonged to the genera Cladosporium, Penicillium, Alternaria, and Aureobasidium. Their presence on the fruit did not depend on the growth conditions of the crop. Fungi that were found only on the organic fruit belonged to the genera Bipolaris, Acremonium, Mucor, and Fusarium. These fungi were not found on fruit that were treated with pesticides.

Fresh organic fruit were inhabited by fungi from 8 different genera, while conventionally cultivated fruit were inhabited by fungi from five different genera. Nevertheless, the Shannon–Wiener diversity index parameters and the number of yeasts and moulds were lower for the fresh organic fruit.

The cold storage of fruits for 8 weeks usually decreased the number of yeasts and moulds and changed the make-up of the dominant genera (the dominant genus was Aureobasidium). Fusarium activity was not eliminated by fruit storage, and zearalenone, which had not been previously detected in fresh fruits, was identified in the fruit samples. In contrast to the conventionally cultivated fruits, the organic fruits were inhabited by fungi of the genus Fusarium and contained deoxynivalenol, a mycotoxin.

Materials and methods
Cultural conditions. The fruits were harvested from ‘Brigitta Blue’ grown on two farms specialized in the cultivation of highbush blueberry, located in the 20 km to the east of Szczecin. The organic cultivation covered 40 ha of land near a peat mine surrounded by pine forests. Blueberry bushes were planted at a spacing of 1.2–2.3 m in 2003. The bushes grew in high peat (Baltic-type) with acidic pH 3.4–3.9, 55.3% organic matter, 5.8% organic carbon, and electrical conductivity 0.27 mS/cm. Every year in autumn and spring, paraffin oil was sprayed. Every year, KALISOP® (50 kg/ha K₂O) and Patentkali® (50 kg/ha K₂O and 20 kg MgO) were applied. The remaining fertilizers annually by means of fertigation, in accordance with the recommendations for highbush blueberry. Ammonium sulfate (200 kg/ha) and the potassium fertilizer KALISOP® (100 kg/ha) were applied. The remaining fertilizers were applied based on soil and leaf analyses. Chemical protection was used as recommended for highbush blueberry. In the leafless period, copper oxychloride spray was sprayed twice. From the beginning of flowering, three sprayings with Signum 33 WG and Switch 62.5 WG were applied. The annual official testing showed that the limit values for pesticide residues in conventional fruit were not exceeded (OJ L70. 16.3.2005).

Every year, during the leafless period, the bushes on both plantations were cut according to the recommendations; approximately 25% of the oldest shoots were cut. In spring, when the temperature dropped below 0 °C, the bushes were sprayed to protect them from spring frosts (Fig. 2). The plantation was irrigated annually using a permanently installed T-Tape drip irrigation line with an emitter performance of 1 L/1 h. The moisture content of the substrates was maintained in the pF 1.8–2.1 range and was determined using contact tensiometers.

Fruit harvest, sample preparation. The fruit was harvested at full ripeness on the basis of an assessment of the colour of the fruit (it must be fully coloured, without a green mark at the stalk) and the total soluble solids—TSS (13–15%). On each plantation, the fruit were collected in two plots (the plot area is approximately 4 ha) from 25 randomly selected bushes in three repetitions (3 × 25 bushes × 2 plots × 2 cultivation methods). The fruits were harvested by hand four times, each time from the same bushes. The fruits were used to prepare the aggregate samples for analysis. The firmness, skin puncture resistance, and colour of the berry were measured in fresh berries immediately after harvest. The heath prompting compounds: phenolic composition, antioxidant activity, α-amylase and α-glucosidase inhibitory activity, were determined in berry samples that were kept frozen.

Fruit and leaf infestation by moulds and yeasts. Branched shoots with leaves, main shoots and fruits were collected at the time of harvesting. The research material was placed in sterile plastic containers. The material was subjected to microbiological analysis (shoots with leaves, main shoots and fruits) immediately after harvesting or after cold storage (blueberry fruits). The analysis of the degree of fruit, shoot and leaf infestation by fungi (yeasts and moulds) was based on the European standard ISO70. After the cultivation of spore-forming fungal inoculates, they were subjected to taxonomic evaluation using the traditional method of macroscopic observation of colonies and microscopic observation of spores and filaments71. To compare the biodiversity of fungi colonizing fruit from both crops, the Shannon–Wiener index was used. The fungi were identified to the genus level. The Shannon–Wiener index is widely accepted in microbial biocenosis monitoring72. Colonies were
counted using an automatic colony counter (Alchem PCC04). Analyses were performed on three replicates from each sample.

**Fruit storage in cold CA storage.** The shock-cooled berries (temperature drop to 3–4 °C within 2 h after picking) were then stored for 8 weeks in a cold room with a controlled atmosphere (CA: CO2-12%; O2-1.5%) at a temperature of 1.5 °C ± 0.25 °C. The experiment was performed in five repetitions, each with 1.25 kg of berries, along with fruits intended for sale. There were approximately 10 tons of fruit in the cold chamber. Each sample was in the chamber only with fruit from the test plots. This was to reduce the chance of infection from pathogens from other plots.

**Colour and firmness.** Measurements were conducted in CIE SCI L*a*b* system—the full nomenclature is 1976 CIE L*a*b* Space, International Commission on Illumination in Vienna [L* white (100) black (0), a* green (− 100) red (+ 100), b* blue (− 100) yellow (+ 100)], through a 10° observer type and D65 illuminant using a KonicaMinolta CM-700d spectrophotometer. The colour parameters and indices were averaged over 35 measurements. The firmness and puncture resistance of the berry skin were measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected berries from three replicates. The result was expressed as a gram-force causing fruit surface to bend 1 mm. Measurements were made on the smaller fruit diameter. Punctures were made using a stamp with a diameter of 3 mm.

**Extraction procedure and identification of polyphenol compounds.** Three replicates of 1000 g randomly chosen blueberries were kept frozen in polyethylene bags at −65 °C until analysis, then prepared according to the methodology of Lachowicz et al.54. The fruits were extracted with methanol acidified with 2.0% formic acid.

**Inhibitory activities and antioxidant activity.** The activity of the fruit extracts was assayed according to the procedure described previously by Podsedek et al.24 (α-glucosidase) and Nickavar and Yousefian25 (α-amylase). All samples were assayed in triplicate, and the result was expressed as the IC50. The amount of the inhibitor (expressed as mg of fruit per 1 mL of reaction mixture under assay conditions) required to inhibit 50% of the enzyme activity was defined as the IC50 value. For the ABTS+ (2,2′-azobis(3-etylbenzotiazolino-6-sulfonian) assay, the procedure followed the method of Arnao et al.76. The FRAP (ferric-reducing antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays were conducted according to the method of Brand-Williams et al.77. The antioxidant capacity was expressed as mmol Trolox/g dw. The ABTS+ and FRAP assay measurements were performed with a UV-2401 PC spectrophotometer. The L-ascorbic acid and nitrate content were measured with an RQflex 10 requantometer (Merck). The content of mycotoxins in blueberry fruits. The samples were purified on AflaTest immunological affinity columns from Vicam for aflatoxins and with OchraPrep from R-Biopharm Rhône Ltd. for ochratoxin A, according to the procedure specified by the manufacturer. Patulin, deoxynivalenol, T2, HT2 toxin and zearalenone were analysed by HPLC–MS/MS. The samples were purified on Bond Elut’ Mycotoxin columns from Varian. Each sample was subjected to three repetitions.

**Statistical analysis.** All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Cracow, Poland). The data were subjected to one-factor ANOVA. Mean comparisons were performed using Tukey’s least significant difference (LSD) test; the significance was set at p<0.05. In addition, the microbial data were ana-
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Author contributions
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Correspondence and requests for materials should be addressed to I.O.

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