

INFLUENCE OF RIPENING TIME ON FRUIT CHEMICAL COMPOSITION OF TWO BLUE HONEYSUCKLE CULTIGENS

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A B S T R A C T

The aim of 3-year experiment was to evaluate quality and chemical composition of blue honeysuckle berries cultivar 'Czarna' and non-specified seedling "N" as dependent on time of ripening. The results obtained showed that berries harvested later were bigger but less firm. Furthermore, an increase of soluble solids content was observed in fruit of both cultigens at the end of harvest (for seedling "N" from 11 to 13.5% and for 'Czarna' from 9.8% to 12.6%). On the other hand, for late-harvested berries of both cultigens 20% decrease of titratable acidity and vitamin C was noticed. In berries of seedling "N" at the beginning of harvest 232 mg 100 g⁻¹ of total polyphenols was determined, whereas the late ripening fruit contained 284 mg 100 g⁻¹ of total polyphenols. Similar relation was observed in 'Czarna' berries (an increase from 164 mg 100 g⁻¹ to 221 mg 100 g⁻¹). The berries of late harvest contained more anthocyanins (167 mg 100 g⁻¹ in 'Czarna' and 227 mg 100 g⁻¹ in seedling "N") as compared to early collected ones (122 and 173 mg 100 g⁻¹ for, 'Czarna' and "N", respectively). Hydroxycinnamic acids content varied from 37.0 to 29.3 mg 100 g⁻¹, respectively, for early and late collected berries of seedling "N" and from 22.3 mg 100 g⁻¹ to 26.6 mg 100 g⁻¹ for early and late harvested berries of 'Czarna'. Among flavonols, quercetin-3-

rutinoside, quercetin-3-glucoside and two unidentified flavonols with retention times 26 min and 32 min, were determined. Luteolin 7-O- α -glucoside content varied from 4.59 to 7.05 mg 100 g⁻¹ in the early and late harvested berries of seedling "N" and from 3.12 mg 100 g⁻¹ in the early to 4.25 mg 100 g⁻¹ in the late harvested berries of 'Czarna'.

Key words: *Lonicera caerulea*, fruit mass and firmness, soluble solids, titratable acidity, L-ascorbic acid, phenolics

INTRODUCTION

The species of *Lonicera* genera are distributed in different zones of northern hemisphere, mostly in moderate-climate regions (Skvortsov and Kuklina, 2002). For the first time blue honeysuckle was mentioned as a horticultural plant in 1894. Blue honeysuckle is also called honeyberry, sweetberry honeysuckle, edible honeysuckle and in Japan haskap or haskappu (Bors, 2008a). By morphological, anatomical, biochemical and DNA analyses, as well as ploidy studies and geographical mapping of blue honeysuckle genetic resources, it has been found that in Eurasia genetic diversity of the crop is represented by four species, of which three: *Lonicera edulis* Turcz ex Freyn, *Lonicera bockarnikowae* Plekh. and *Lonicera iliensis* Pojark are the endemic diploids and one, *Lonicera caerulea* L., is a tetraploid. Only *L. caerulea* has been domesticated (Plekhanova, 1995). According to Bieniek et al. (2005), edible-fruit bearing varieties descent from *L. caerulea*: *Lonicera caerulea* var. *edulis* Reg., *L. caerulea* var. *tanganica* Max., *L. caerulea* var. *kamtschatica* Sevest. and *L. caerulea* var. *altaica* Pall. Major advantageous

features of blue honeysuckle are extra-early ripening, high content of ascorbic acid and bioactive flavonoids in fruit, and outstanding frost resistance of plants and flowers (Plekhanova, 2000). The blue honeysuckles ripen weeks before strawberries and have a flavour commonly described as a combination of blueberries and raspberries. The plants bear at a very young age and the fruit are easily shaken off at harvest time. They may be ideally suited for mechanized harvesting since they do not sucker and have bushes of a similar size to other fruit plants that are harvested by machines. The plants appear to have a few pests and diseases making it a worthwhile crop to consider for organic production. Honeysuckle berries have been used in a wide range of products including juices, wines, candies, pastries, jams, dairy products and are eaten fresh (Bors, 2008b). The *Lonicera* berries exhibit anti-inflammatory and antibacterial activities and thus have a beneficial effect on human organism (Park et al., 2005; Jin et al., 2006). In Poland, a growing interest has been observed in blue honeysuckle cultivation and breeding.

The purpose of the study was to evaluate fruit quality as well as

chemical composition (soluble solids, L-ascorbic acid and phenolics content and titratable acidity) of early and late collected berries of blue honeysuckle cultivar 'Czarna' and non-specified seedling "N".

MATERIAL AND METHODS

The experiment was undertaken at the Experimental Station at Rakowo near Szczecin in 2006-2008. The randomized block experimental design was used with three replications; each consisted of four bushes per a plot. Blue honeysuckles were planted in the spring of 2005 in brown podsolic soil (IIIrd valuation class) rich in the nutrients and cultivated under conventional agronomic treatments adequate to low soil and water requirements of the species. Because *Lonicera* plants are resistant to diseases "by nature", no chemical protection was applied. Each year, fully coloured berries were collected several times following their ripening. For comparison of early and late ripening berries of both genotypes, the measurements were performed on berries of 1st and 2nd harvest and of two last harvests. The fruit mass, firmness, soluble solids and vitamin C contents and titratable acidity were measured on fresh berries, whereas phenolics composition and content was determined on fruit stored at -32°C in polyethylene bags.

Immediately after the harvest, fruit mass was measured with RADWAG WPX 4500 electronic scales (0.01 g accuracy) and fruit firmness was measured with a Firm-

Tech 2 apparatus (BioWorks, USA) on 100 randomly selected berries from each replication. The firmness was expressed as a force (in grams) causing fruit surface to bend by 1 mm. Titratable acidity was determined by titrating water extract of berry homogenate with 0.1 N NaOH to the end point of pH 8.1 (measured with an Orion 720 A pH meter; Orion Research Incorporated, USA) according to PN-90/A-75101/04. Soluble solids content was determined with an Abbé refractometer (PN-90/A-75101/02). Vitamin C content was measured with RQflex 10 reflectometer (Merck) and expressed as mg per 100 g of fruit tissue. For the HPLC analyses, 2 g aliquots of fruit were extracted three times with approx. 8 ml of 80% MeOH acidified with acetic acid (1 ml of 100% acetic acid per 1 l 80% MeOH) in an ultrasonic bath for 15 min. The samples were filtered, transferred to the volumetric flasks and made up to the final volume of 25 ml. Then, the extracts were centrifuged twice at 12,000 g and 20 µl of supernatants were injected into the HPLC system. The separation was performed on Cadenza CD C18 column (75 x 4.6 mm, 5 µm), (Imtakt, Japan). Column oven temperature was set at 30°C. The mobile phase was composed of solvent A (4.5% formic acid, pH 2.2) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 21% B (0-30 min), followed by washing and reconditioning the column. The flow rate was 1 ml min⁻¹ and the runs were monitored at the following wave-

lengths: phenolic acids at 320 nm, flavonols and luteolin derivative at 360 nm, and anthocyanin glycosides at 520 nm. The Photo Diode Array spectra were measured over the wavelength range 200-600 nm with the resolution of 2 nm. Retention times and spectra were compared to these of pure standards and phenolics content was expressed as mg per 100 g of fresh matter. Standards of anthocyanidin glycosides were obtained from Polyphenols Laboratories (Norway), while for phenolic acids, flavonols and luteolin 7-O- α -glucoside from Extrasynthese (France).

The results obtained were subjected to statistical analysis using Statistica 8.0 software (Statsoft, Poland). The analysis of variance in a form of 3-year synthesis for fixed model was used. The mean values were evaluated by the Duncan test and for phenolics by the Student t-test. The differences at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The plants blossomed at the end of April and early fruit were harvested in the mid of May at air temperature from 11°C to 13°C. The late fruit ripened 3 weeks later when air temperature was a few centigrades higher. At the end of harvest, the mass of one fruit for seedling "N" was 17% higher than at the beginning, whereas for 'Czarna' by 13%. However, independently of the time of harvest, berries of seedling "N" were smaller than 'Czarna' fruit

(Tab. 1). Arus and Kask (2007) reported that the mass of honeysuckle berries varies from 0.5 g to 2.0 g. However, some Russian cultivars have small berries (0.5 g), whereas some Japanese cultivars achieve 2-2.4 g.

The increase in fruit mass was accompanied by enhancement of fruit dimensions. Both diameter and length of fruit significantly increased at the end of cropping. The studies carried out by Ścibisz et al. (2003) on highbush blueberry and by Ochmian et al. (2007a) on strawberry showed that later ripening berries had significantly smaller fruit. Different trend in blue honeysuckle may have resulted from higher temperatures later in the season. Thus, the fruit collected earlier grew in less favourable weather conditions.

On the other hand, the time of harvest had a disadvantageous effect on blue honeysuckle fruit firmness. The early harvested fruit were harder compared to late collected ones, and 'Czarna' berries showed higher firmness compared to seedling "N" fruit. However, the results obtained for other species show that along with an enhancement of fruit dimension a decrease of firmness is commonly observed (Ochmian et al., 2007b).

In this study, soluble solids content varied from 9.8 to 13.5% (Tab. 2) and it was in accordance with data obtained by Kamzolova et al. (2006) for 15 honeysuckle cultivars (9.1-12.6%). At any time of harvest, the berries of seedling "N" had higher soluble solids content than those of 'Czarna'. For both cultivars, late berries which ripened at higher

Table 1. Fruit quality of two blue honeysuckle genotypes (an average for 2006-2008)

Characteristics	Time of measurement	Cultigen	
		seedling "N"	'Czarna'
Weight of 1 fruit [g]	harvest beginning	0.78±0.05 a/A***	1.17±0.04 a/B
	end of a harvest	0.94±0.02 b/A	1.34±0.02 a/B
Fruit diameter [mm]	harvest beginning	8.2±0.4 a/A	11.0±0.5 a/B
	end of a harvest	10.4±0.2 b/A	13.5±0.2 b/B
Fruit length [mm]	harvest beginning	18.6±0.4 a/B	15.8±0.4 a/A
	end of a harvest	22.1±0.1 b/B	18.8±0.2 b/A
Firmness [G·mm ⁻¹]	harvest beginning	153±14 b/A	182±18 b/B
	end of a harvest	115±21 a/A	126±23 a/B

*Values are the mean of three determinations ±standard deviation and refer to freshly harvested fruit

**Different letters in the same row indicate significant differences at $p < 0.05$. Lower case refer to harvest date (for a particular cultivar) whereas capital letters refer to comparisons of two genotypes

Table 2. Soluble solids, titratable acidity and vitamin C contents in honeysuckle fruit depending on time of harvest (an average for 2006-2008)

Characteristics	Time of measurement	Cultigen	
		seedling "N"	'Czarna'
Soluble solids [%]	harvest beginning	11.1±0.3 a/B***	9.8±0.3 a/A
	end of a harvest	13.5±0.2 b/A	12.6±0.2 b/A
Titratable acidity [g citric acid·100g ⁻¹]	harvest beginning	3.55±0.31 b/B	2.74±0.23 b/A
	end of a harvest	2.79±0.18 a/A	2.28±0.16 a/A
L-ascorbic acid [mg·100g ⁻¹]	harvest beginning	105±15 b/B	74±8 b/A
	end of a harvest	78±12 a/B	50±5 a/A

*, **Explanations, see Table 1

temperatures had significantly higher content of soluble solids. Poll and Petersen (2003) observed that sour cherries obtained in a cold and rainy season showed soluble solids concentration 16-18 °Brix whereas, in the years of higher temperatures

26-28 °Brix. Titratable acidity, calculated as citric acid equivalent, varied from 2.28 g 100 g⁻¹ in 'Czarna' berries to 3.55 g 100 g⁻¹ in fruit of seedling "N" (Tab. 2). For another Polish cultivar 'Zielona', titratable acidity, expressed as citric acid, was 2.98 g

100 g⁻¹ (Skupień et al., 2007), whereas Kamzolova et al. (2006) determined that titratable acidity, expressed as malic acid, ranged from 1.79% to 3.24%.

Contrary to soluble solids, the berries of both cultivars collected at the end of harvest time showed significantly lower acidity. Thus, blue honeysuckle berries which ripened later had much better taste due to the increased soluble solids content and decreased acidity. The soluble solids : titratable acidity ratio for seedling “N” increased from 3.1 : 1 in early ripening berries to 4.8 : 1 in late ripening ones whereas for ‘Czarna’ from 3.6 : 1 to 5.5 : 1, respectively.

Fruit of seedling “N” surpassed ‘Czarna’ berries regarding L-ascorbic acid content (105.0 mg 100 g⁻¹ for early collected berries and 78.0 mg 100 g⁻¹ for late ripening ones as compared with 74 and 50 mg 100 g⁻¹, respectively, for ‘Czarna’) (Tab. 2). Kamzolova et al. (2006) reported that ascorbic acid content in blue honeysuckles grown in Belarus varied from 28.0 to 48.0 mg·100 g⁻¹, which is significantly less than contents determined for both genotypes in this research. Pierzga (2001) also reports lower content of vitamin C (25 mg 100 g⁻¹) in fruit of ‘Czelabińska’ selection.

The phenolic profiles and content in berries of both cultivars are presented in Figure 1 and Table 3. Total phenolics content observed in this study varied from 164.22 mg 100 g⁻¹ (early collected berries of ‘Czarna’) to 284.28 mg 100g⁻¹ (late harvested

berries of seedling “N”). Skupień et al. (2007) reported that total phenolics content in ‘Zielona’ fruit amounted to 319.11 mg 100 g⁻¹. The anthocyanins accounted for 74.4-79.8% of total phenolics in berries of seedling “N” and 74.6-75.3% in ‘Czarna’ fruit. Similar participation of anthocyanins was found in ‘Zielona’ fruit by Skupień et al. (2007). The major anthocyanin in both blue honeysuckle cultivars was cyanidin 3-glucoside (103.06-190.04 mg 100 g⁻¹, 62-84% of total anthocyanin content) followed by cyanidin 3-rutinoside (3.15-23.40 mg 100 g⁻¹), cyanidin 3,5-diglucoside (8.66-14.96 mg 100 g⁻¹), and peonidin 3-glucoside (2.54-9.36 mg 100 g⁻¹). Oszmiański et al. (1995) found that cyanidin 3-glucoside accounted for 91% of the total anthocyanins in blue honeysuckle. On the other hand, Chaovanalikit et al. (2004) analyzing 10 genotypes of blue honeysuckle identified additionally peonidin 3-rutinoside and pelargonidin 3-glucoside. In this study, the berries of late harvest, which ripened at higher temperatures, had higher content of anthocyanins and total polyphenols. Wang and Zheng (2001) have found that the total content of pelargonidin-3-glucoside and cyanidin-3-glucoside in strawberries grown in cool day and night temperatures (18/12°C) was 309.8 µg g⁻¹ in ‘Earliglow’ and 391.7 µg g⁻¹ in ‘Kent’, whereas at temperatures 30/22°C the pigment content increased to 782.7 and 990.9 µg g⁻¹ in ‘Earliglow’ and ‘Kent’, respectively. Both honeysuckle cultivars showed different values of

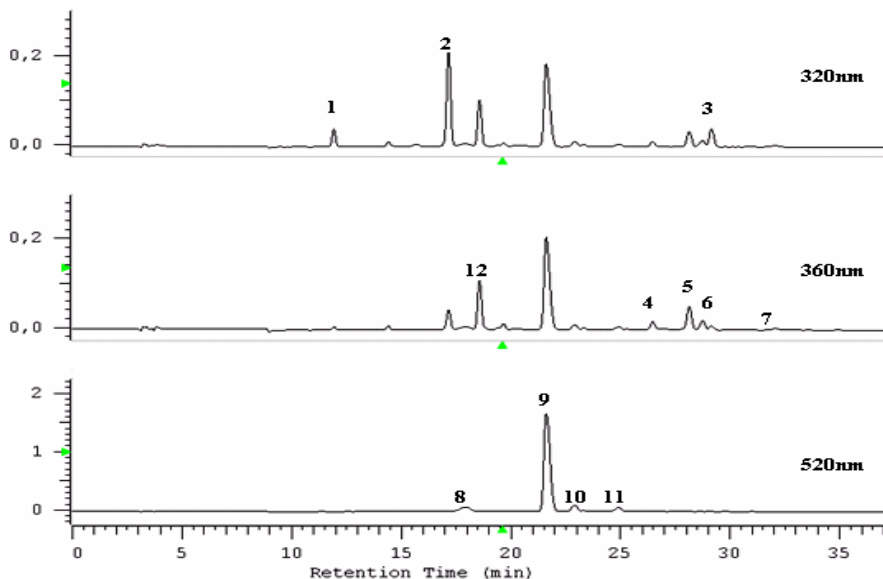
Table 3. Fruit phenolics content of two honeysuckle genotypes depending on harvest time (an average for years 2006-2007)

Compound	Phenolics content [mg·100 g ⁻¹]			
	seedling "N"		'Czarna'	
	time of harvest			
	harvest beginning	end of a harvest	harvest beginning	end of a harvest
Neochlorogenic acid	2.97±0.12 a* **	3.20±0.81 b	0.41±0.02 a	0.42±0.03 a
Chlorogenic acid	28.29±1.78 b	21.71±1.15 a	19.57±1.24 a	23.48±0.98 b
3,5-dicaffeoyl quinic acid	5.76±0.37 b	4.35±0.41 a	2.31±0.25 a	2.67±0.29 a
Sum of hydroxycinnamic acids	37.02 b¹/B²	29.26 a¹/A	22.29 a/A²	26.57 a/A
Unidentified flavonol (retention time 26 min)	1.63±0.11 a	2.53±0.19 b	4.07±0.22 a	6.22±0.25 b
Quercetin 3-rutinoside	14.04±0.74 a	16.17±0.69 b	8.10±0.48 a	12.10±0.59 b
Quercetin 3-glucoside	1.00±0.08 a	1.22±0.12 a	2.13±0.12 a	2.72±0.16 b
Unidentified flavonol (retention time 32 min)	1.09±0.14 a	1.25±0.16 a	2.02±0.21 a	2.91±0.24 b
Sum of flavonols	17.76 a/A	21.17 a/A	16.32 a/A	23.95 b/B
Cyanidin 3-5-diglucoside	8.93±0.37 a	10.04±0.25 a	8.66±0.31 a	14.96±0.55 b
Cyanidin 3-glucoside	145.52±16.2 a	190.04±13.8 b	103.06±12.4 a	137.95±11.0 b
Cyanidin 3-rutinoside	15.85±1.02 a	23.40±0.59 b	3.15±0.24 a	4.74±0.33 a
Peonidin 3-glucoside	2.54±0.19 a	3.33±0.23 a	7.62±0.41 b	9.36±0.55 a
Sum of anthocyanins	172.84 a/B	226.81 b/B	122.49 a/A	167.01 b/A
Luteolin 7-O- α -glucoside	4.59±0.35 a	7.05±0.29 b	3.12±0.11 a	4.25±0.41 a
Total	232.20 a/B	284.28 b/B	164.22 a/A	221.79 b/A

*: **Explanations, see Table 1

cyanidin 3-glucoside : cyanidin 3-rutinoside ratio. Moreover, during cropping season a slight decline of cyanidin 3-glucoside : cyanidin 3-rutinoside ratio was observed; in fruit of seedling "N" it was 9.2 : 1 at the beginning of harvest and 8.1 : 1 at the end whereas in berries of 'Czarna' it was 33 : 1 and 29.1 : 1, respectively.

Berries of "N" seedling contained higher amounts of chlorogenic acid (with exception of the late harvest), 3,5-dicaffeoyl quinic acid and neochlorogenic acid than 'Czarna' fruit. The total content of hydroxycinnamic acids ranged from 22.29 to 37.02 mg 100 g⁻¹ and was lower than that reported by Chaovanalikit et al.



- | | |
|---|---|
| 1. Neochlorogenic acid | 7. Unidentified flavonol (r. t. 32 min) |
| 2. Chlorogenic acid | 8. Cyanidin 3-5-diglucoside |
| 3. 3,5-dicaffeoyl quinic acid | 9. Cyanidin 3-glucoside |
| 4. Unidentified flavonol (r. t. 26 min) | 10. Cyanidin 3-rutinoside |
| 5. Quercetin 3-rutinoside | 11. Peonidin 3-glucoside |
| 6. Quercetin 3-glucoside | 12. Luteolin 7-O- α -glucoside |

Figure 1. HPLC profile of phenolics in blue honeysuckle fruit

(2004) (30.4-156 mg 100 g⁻¹, expressed as chlorogenic acid). For late ripening berries of seedling “N” a significant decrease (by 21%) of hydroxycinnamic acid content was observed whereas 19% enhancement was found in ‘Czarna’ fruit. The changes resulted mostly from alterations in chlorogenic acid content.

The luteolin 7-O- α -glucoside was identified in both cultivars, however the levels determined in berries of the seedling “N” exceeded these of ‘Czarna’. For both cultivars, late ripening berries showed an increased level of this flavon, amounting to 7.05 mg 100 g⁻¹ for seedling “N” and

4.25 mg 100 g⁻¹ for ‘Czarna’. In our previous study (Skupień et al., 2007), the quantity of luteolin 7-O- α -glucoside found in berries of ‘Zielona’ cv. was higher (9.40 mg 100 g⁻¹). Streltsyna et al. (2006) reported luteolin glycosides content varying from 1.5 to 20.7 mg 100 g⁻¹ in 51 genotypes of honeysuckle grown in Russia.

Both cultivars studied in this experiment had a similar level of total flavonols in the fruit, ranging from 16.32 to 23.95 mg 100 g⁻¹, with higher content in the late ripening berries. Chaovanalikit et al. (2004) have found that the content of fla-

vonols in blue honeysuckle fruit ranged from 14.0 to 32.8 mg 100 g⁻¹ (calculated as quercetin 3-rutinoside) whereas in 'Zielona' fruit it was 17.15 mg 100 g⁻¹ on average (Skupień et al., 2007). Quercetin 3-rutinoside was found to be a predominant compound, constituting 50-79% of total flavonols. Streltsyna et al. (2006) determined quercetin 3-rutinoside (rutin) content in Russian honeysuckle cultivars varying from 0 to 20.6 mg 100 g⁻¹. The content of quercetin 3-glucoside in 'Czarna' fruit (2.13 and 2.72 for early and late ripening berries, respectively) was twice as high as that in fruit of the seedling "N" (1.00 and 1.22 for early and late berries, respectively). Streltsyna et al. (2006) determined that the amount of quercetin 3-glucoside (isoquercetin) in blue honeysuckle fruit varied from 2.4 mg 100 g⁻¹ to 18.7 mg 100 g⁻¹. In this study, the peaks 4 and 7, eluted respectively at 26 and 32 min, had the spectra similar to those of flavonols and were assigned as the unidentified flavonols summing up to total flavonol content (Fig. 1).

CONCLUSIONS

1. On 3-year average, higher fruit mass and diameter had blue honeysuckles of 'Czarna' cv., while harder and longer berries had seedling "N". For both cultivars, later ripening berries were bigger but showed decreased firmness.
2. Throughout the cropping season, the berries of seedling "N" were characterized by higher content of soluble solids, titratable acidity, L-ascorbic acid, and polyphenolic

compounds (especially anthocyanins, hydroxycinnamic acids, and luteolin 7-O- α -glucoside) compared to 'Czarna' fruit.

3. The late ripening berries of both cultivars showed an enhanced level of soluble solids and total polyphenols accompanied by a decrease of titratable acidity and L-ascorbic acid content. Moreover, the berries of both cultivars collected at the end of harvest season had better taste due to an increased soluble solids content and decreased titratable acidity.

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WPŁYW TERMINU ZBIORU NA SKŁAD CHEMICZNY DWÓCH ODMIAN JAGODY KAMCZACKIEJ

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STRESZCZENIE

Celem 3-letniego doświadczenia była ocena jakości i składu chemicznego owoców jagody kamczackiej odmiany 'Czarna' oraz genotypu o nieznanym pochodzeniu (siewka "N"). Stwierdzono, że owoce zbierane później były większe, ale miały obniżoną jędrność. W owocach obydwu odmian pod koniec zbioru obserwowano wzrost zawartości ekstraktu (dla siewki "N" z 11 do 13,5%, a dla owoców odmiany 'Czarna' z 9,8 do 12,6%). Z drugiej strony, owoce obydwu odmian zbierane później wykazywały 20% spadek kwasowości i witaminy C. Zawartość polifenoli ogółem w jagodach siewki "N" na początku zbiorów wynosiła 232 mg 100 g⁻¹, a pod koniec zbiorów 284 mg 100 g⁻¹. Podobną relację obserwowano w owocach odmiany 'Czarna' (wzrost ze 164 mg 100 g⁻¹ do 221 mg 100 g⁻¹). Owoce zbierane później miały większą zawartość antocyjanów: 167 mg 100 g⁻¹ ('Czarna') i 227 mg 100 g⁻¹ (siewka "N") w porównaniu z odpowiednio 122 i 173 mg 100 g⁻¹ dla owoców zbieranych wcześniej. Zawartość kwasów hydroksycynamonowych wahała się od 37,0 mg 100 g⁻¹ do 29,3 mg 100 g⁻¹ (odpowiednio dla wcześniej i później zbieranych owoców nieokreślonego genotypu), natomiast dla wcześniej i późno zbieranych owoców odmiany 'Czarna' odpowiednio od 22,3 mg 100 g⁻¹ do 26,6 mg 100 g⁻¹. Spośród flawonoli stwierdzono obecność kwercetyno 3-rutynozydu i kwercetyno 3-glukozydu oraz dwóch niezidentyfikowanych związków o czasach retencji 26 i 32 min. Zawartość luteolino 7-O- α -glukozydu wynosiła od 4,59 mg 100 g⁻¹ do 7,05 mg 100 g⁻¹ (dla odpowiednio wcześniej i późno zbieranych jagód siewki "N") oraz od 3,12 mg 100 g⁻¹ do 4,25 mg 100 g⁻¹ dla owoców odmiany 'Czarna'.

Słowa kluczowe: *Lonicera caerulea*, masa i jędrność owoców, ekstrakt, kwasowość, witamina C, polifenole