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Original scientific article

FRUIT MATURITY STAGE IN RELATION TO CONTENT OF POLYPHENOLS, FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF SELECTED CLONES OF *Lonicera kamtschatica* (Sevast.) Pojark

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The article is dealing with evaluation of four fruit maturity stages of 6 selected clones (LKL-3, LKL-14, LKL-19, LKL-33, LKL-96 and LKL-103) of Lonicera kamtschatica (Sevast.) Pojark in relation to total polyphenols, flavonoids content and antioxidant activity of berries. The experiment in 5 replications of each clones has been established in the conditions of Botanical Garden in Nitra (Slovak republic)in 2016. The results of analyses proved that in respect of the total polyphenols content (TPC), the content of flavonoids (FC) and the antioxidant activity the most valuable clones at the stage of full ripened fruit were following: LKL-14 (8.77; 2.57 g/kg FW; 9.07 g/kg), LKL-33 (6.77;

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2.84 g/kg FW; 9.16 g/kg) and LKL -3 (8.19; 2.61 g/kg FW; 8.94 g/kg). The results of statistical analyses by one-way analysis of variance proved significant differences among assayed clones in total flavonoids and antioxidant activity of fruit but no differences among content of total polyphenols and assayed stages of fruit maturity.

Keywords: *Lonicera*, antioxidants, polyphenols, fruits

INTRODUCTION

Minor or underutilized fruits are easier to grow and hardy in nature, producing a crop even under adverse soil and climatic conditions. Their fruits has unique aroma and taste and are playing a vital role in nutrition and livelihood in particular for rural and tribal masses for employment and income generation (VIJAYAN *et al*., 2008; MIKULIC-PETKOVSEK *et al*., 2012; TSUKAMOTO *et al*., 2018)

The edible honeysuckle belongs to the group of valuable underutilized fruit species because of the high content of bioactive compounds (JURÍKOVÁ *et al*., 2012; JURÍKOVÁ *et al*., 2014). The berries are rich in ascorbic acid, macro, microelements and polyphenolic compounds (FREJNAGEL, 2007; ROP *et al*., 2011; JURIKOVA *et al*., 2012). According to research work of JURÍKOVÁ *et al.* (2012), KUCHARSKA *et al*. (2017) and MECH-NOWAK *et al.* (2014) the berries are rich in compounds from two groups: monoterpenes (iridoids) and polyphenols (anthocyanins, flavonols, flavanonols, flavones, flavan-3-ols, phenolic acids-chlorogenic, ferulic, salicyl, cinnamic, and caffeic acid). Cyanidin-3-glucoside can be considered as the major compound amomg polyphenols (JURÍKOVÁ *et al*., 2012; CHEN *et al*., 2014;). The berries are effective in improvement of chronic diseases on the base of inflammation (JURÍKOVÁ *et al*., 2012; WU *et al*., 2015) such as cancer (PALÍKOVÁ *et al*., 2009), metabolic disorders and diabetes mellitus (JURGONSKI *et al*., 2013; PODSEDEK *et al*., 2014), cardiovascular diseases, hyperthyroidism (PARK *et al*., 2016) and arthritis (WU *et al*., 2015).

The content of the major bioactive compounds in the edible honeysuckle berries has been determined in relation to species, cultivar (variety) (WOJDYLO *et al*., 2013; JURÍKOVÁ *et al*., 2014), conditions of cultivation, fertilizers (SZOT *et al*., 2012), irrigation (JURÍKOVÁ *et al*., 2007; JURÍKOVÁ *et al*., 2009), different methods of extraction and detection (GAZDÍK *et al*., 2009; KUSZNIEREWICZ *et al*., 2012; ZHAO *et al*., 2015; CELLI *et al*., 2015) and preparation forms (powder, juice (KOSS-MIKOLAJCZYK et al., 2015; OSZMIANSKI *et al.*, 2016).

There are only few research sources dealing with changes in nutritional value of fruit in relation to fruit development and ripening stages. Study of JURÍKOVÁ *et al.* (2009) proved that different development stages has significant influence on sugar and organic acids content. According to authors in the stage of fruit softening was achieved the best quality for direct consumption; characterized by the highest sugar content (9.5% in *Lonicera kamtschatica* and 7.7% in *Lonicera edulis*) and the lowest organic acids content (3.61% in *Lonicera kamtschatica* and 3.32% in *Lonicera edulis*). In another study OCHMIAN *et al.* (2012) compared the fruit quality and chemical composition of *Lonicera kamtschatica* cultivars but only at the beggining and end of the harvested season. The results of experiments showed that ´Wojtek´ displayed the higher level of total polyphenols 183.66 mg/100 g at the stage of late ripening fruit.

The blue honeysuckle (*Lonicera kamtschatica, Lonicera edulis*) is quite early ripening species in Slovakia (JURÍKOVÁ *et al*., 2009) and Poland (SKUPIEN *et al*. 2007) and could be a

good first source of vitamins and anthocyanins after long winter period. For this reason it is very important to state the optimal term of fruit harvesting in respect to the highest level of dominated bioactive compounds - polyphenols. Moreover, the research study was aimed at study of changes in level of total polyphenols, flavonoids and antioxidant activity at 4 stages of fruit maturity for *Lonicera kamtschatica* (Sevast.) Pojark-selected 6 clones (LKL-3, LKL-14, LKL-19, LKL-33, LKL-96, LKL-103).

MATERIAL AND METHODS

Plant material

The assayed biological material was originated from Botanical garden of Slovak University of Agriculture in Nitra. The basic genofond of university – *Lonicera kamtschatica* (Sevast.) Pojark and *Lonicera edulis* Turcz. Ex. Frey was in 2002 extended by further 27 clones of *Lonicera kamtschatica* (Sevast.) Pojark. Gerda/25 selected and gifted by Herbaton Klčov s.r.o. Each clone was presented in 6 replications. From this genofond 6 clones were selected according to different time of ripening and morphological properies (LKL-3, LKL-14, LKL-19, LKL-33, LKL-96 and LKL-103). The soil and climatic conditions of the position were as follows: open level, 130 m above sea level, corn growing region, clay-loam-drift, pH 6.4, mould content of 3.5%, precrop black fallow, average rainfall of 564 mm per year, the average temperature in vegetation period of 16.3°C. The mean air temperatures and rainfal in 2016 are given in the Table 1.

Table 1. Monthly mean temperatures and rainfalls

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
temperatures °C	-0.6	5.5	4.3	9.3	15.0	20.3	21.4	19.5	17.5	9.4	3.8	-2.7
rainfall mm	29	78	32	25	91	14	135	35	37	69	35	14

The fruit development stages was divided into 4 stages corresponding with skin colour and fruit softening; immature, mid-ripe, ripe, and overripe fruit.
immature - enlargement of fruit pericarp and intensive fruit colouring (stage I)
mid-ripe - fruit softening (stage II)
full ripened fruit (stage III) - fruit were uniformly coloured and softened and they were easily separable from stalk over – ripe fruit (stage IV).
The fruits were taken from 6 clones of Lonicera kamtschatica, each clone was presented in 5 repetitions. The average sample of clone represented 10 g.

The first berry samples were taken after begining of berries colouring. In immature fruit ripening process proceeded (80% blue and 20% pale green skin) and were undergoing cell enlargement, mid-ripe fruit had the typical skin colour i.e., uniformly blue with waxy coating, ripe fruit were softened and easily separable from pedicle. Overripe fruit was characterized by shrinkage due to water loss. Fruit of individual cultivars were processed immediately after the harvest (not later than within two days). Harvested fruit were puréed in a mixer and the average sample was obtained by dividing into quarters.

The measurement of total phenolic content (TPC)

Extraction and total phenolic content assay were performed according to the method described by KIM *et al.* (2003), using the following procedure: 10 g of fresh sample were homogenized for 10 s in 100 ml of methanol. The resulting paste was placed into Erlenmeyer flasks (120 ml) and let to stand in a water bath with the temperature of 25°C for a period of 24 h. The residue was then extracted with two additional portions of methanol. The combined methanolic extracts were evaporated at 40°C to dryness and redissolved in methanol at a concentration of 100 mg/ml, and stored at 4°C for further use. To measure total contents of phenolic substances, 0.5 ml of the sample was taken and diluted with water in a 50-ml volumetric flask. Thereafter, 2.5 ml of Folin-Ciocalteau reagent and 7.5 ml of a 20-percent solution of sodium carbonate were added. The resulting absorbance was measured in the spectrophotometer LIBRA S6 (Biochrom, Ltd., Cambridge, UK) at the wavelength of 765 nm against a blind sample, which was used as reference. The results were expressed as g of gallic acid/kg of fresh mass (FM).

The measurement of total flavonoid content (TFC)

The total flavonoid content was determined following SINGLETON *et al.* (1999). In a 10-ml Eppendorf tube, 0.3 ml of the fruit extract, 3.4 ml of 30% ethanol, 0.15 ml of $NaNO_2$ (c = 0.5 mol/l) and 0.15 ml of $AlCl_3 . 6H_2O$ (c = 0.3 mol/l) were added and mixed. After 5 min, 1 ml of NaOH (c = 1 mol/l) was added, and the mixture was measured at the wavelength of 506 nm. The total flavonoid concentration was calculated from a calibration curve using rutin as the standard. The results were expressed in g/kg FM.

The measurement of antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) was done according to the method of THAIPONG *et al.* (2006). The stock solution was prepared by dissolving 24 mg of DPPH with 100 ml of methanol and then stored at –20°C until needed. The working solution was obtained by mixing 10 ml of stock solution with 45 ml of methanol to obtain the absorbance of 1.1 ± 0.02 units at 515 nm using the spectrophotometer LIBRA S6. Fruit extracts (150 µl) were allowed to react with 2,850 µl of the DPPH solution for 1 h in the dark. Then the absorbance was taken at 515 nm. The antioxidant activity was calculated as a decrease in absorbance value using the equation: Antioxidant activity (%) = (A0-A1/A0) × 100 where: A0 – absorbance of the control (without the sample) A1 – absorbance of the mixture containing the sample. The results of absorbance were converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) (RUPASINGHE *et al.*, 2006). For comparison, the extract providing 50% of radical scavenging activity (IC50, the concentration of the sample to scavenge 50% of the DPPH radicals) was calculated from the graph of radical scavenging activity percentage against extract concentration. For this purpose, dilution series (five different concentrations) were prepared for each cultivar extract. The results were calculated and expressed in µg/ml.

Statistical analysis

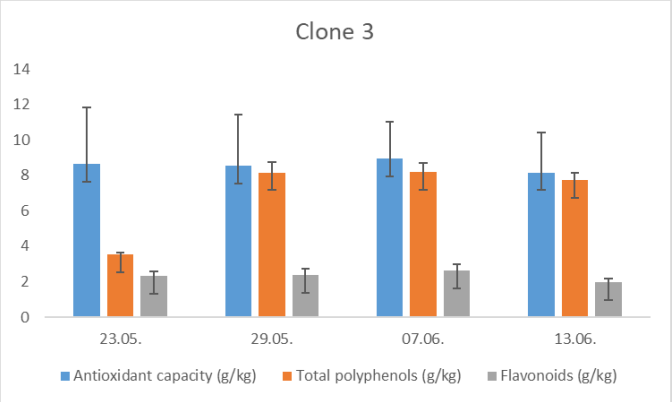
Results of research were tested by one-way analysis of variance evaluated the clones and date of sample as source of variability.

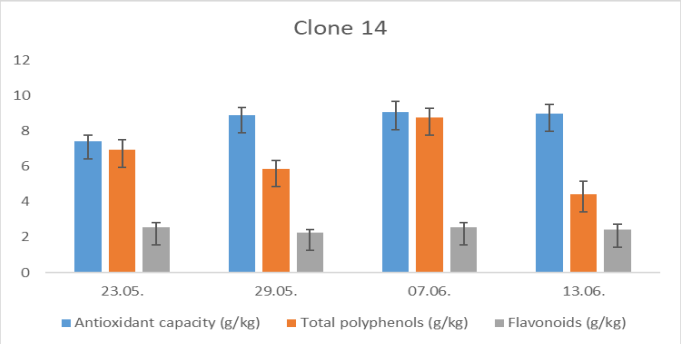
RESULTS AND DISCUSSION

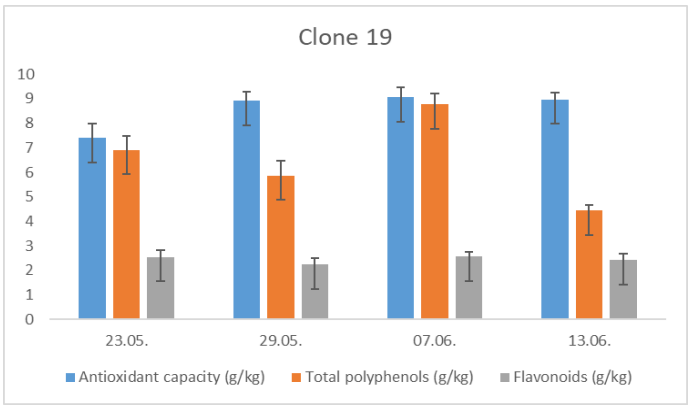
First of all, in our research we focused attention to observation of fruit development stages. According of the study SZOT-WIENERSKA (2012) from the appearance of the typical colour to fully ripened fruit lasted 5-10 days. In our study from mid-ripe fruit up to ripe lasted 8 days. Similarly, fruit ripening process occurred in condition of Slovakia and Poland in May (ARUS-KASK, 2007). In our research fully ripened fruit were yielded in the first decade of June that corresponded with observation of Malodobry *et al.* (2008). Ripening of fruit and their quality are strongly dependent on the weather conditions during vegetation period as it was reported by (SZOT-WIENERSKA, 2012; JURÍKOVÁ *et al.*, 2012). Upon the beginning harvesting, LKL-3, LKL-14, LKL-19 and LKL-33 can be regarded as an earlier clones than LKL-96 and LKL-103.

Secondly, the berries of selected clones were analysed in the total polyphenols, flavonoids and antioxidant activity in relation to different stages of fruit maturity.

The results of experiments showed that the tested clones can be characterized by different levels of total polyphenols, flavonoids, and antioxidant activity of fruit as it was demonstrated in Figure 1-6 .






Clone 19
10
9
8
7
6
5
4
3
2
1
0
23.05.
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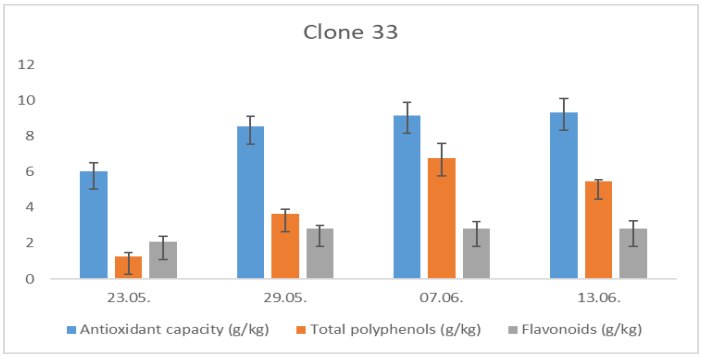
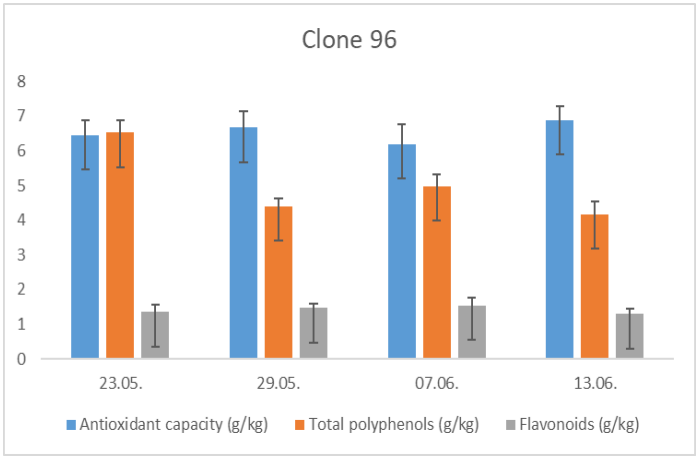
Antioxidant capacity (g/kg)
Total polyphenols (g/kg)
Flavonoids (g/kg)


Clone 33
12
10
8
6
4
2
0
23.05.
29.05.
07.06.
13.06.
Antioxidant capacity (g/kg)
Total polyphenols (g/kg)
Flavonoids (g/kg)


Clone 96
8
7
6
5
4
3
2
1
0
23.05.
29.05.
07.06.
13.06.
Antioxidant capacity (g/kg)
Total polyphenols (g/kg)
Flavonoids (g/kg)

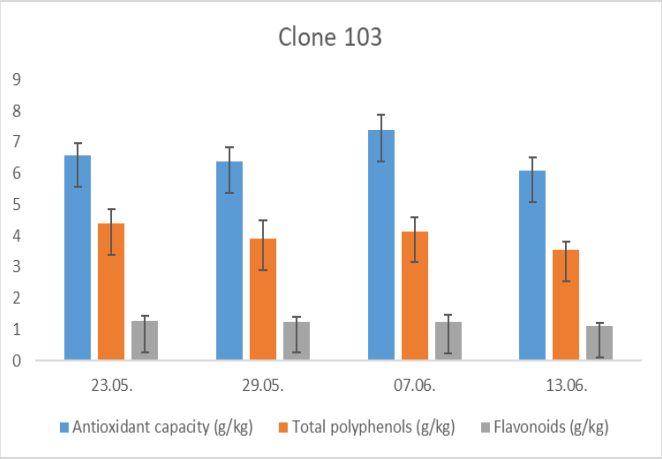


Fig. 1-6 The total polyphenols, flavonoids content and antioxidant activity of selected 6 clones of *Lonicera kamtschatica* (Sevast.) Pojark in different stages of fruit maturity

Statistical analyses proved statistically significant differences in antioxidant activity (p=0.002) and flavonoids content (p=0.001) among assayed clones of *Lonicera kamtschatica* (Sevast.) Pojark. On the other hand, differences between evaluated stages of maturity have not been confirmed as statistically significant (antioxidant activity p=0.316; polyphenols p=0.240; flavonoids p=0.924). This results are in conflict with studies PLEKHANOVA (1990) and JURÍKOVÁ *et al*. (2012) pointed to high variability in polyphenols content of the edible honeysuckle fruit in respect to species and cultivar (genotypes). Disrepancies can be explained by different type of biological material (genotype) and climatic and soil conditions as well (JURÍKOVÁ *et al*., 2012). The content of flavonoids in the berries of edible honeysuckle is statistically significantly dependent on species (cultivars) (PETROVA, 1986; JURÍKOVÁ *et al*., 2012). According to research of OCHMIAN *et al.* (2012) the total phenol and flavonoids content for honeysuckles was also cultivar- and harvest-date-dependent.

On the basis of achieved results we can observe the different dynamic of fruit ripening in relation of assayed clones. LKL-3, LKL-4, LKL-19 and LKL-33 achieved the highest level of total polyphenols at the stage of fully riped fruit, on the contrary, LKL-96 and LKL-103 at the begging of the process of fruit ripening. On contrary, OCHMIAN *et al.* (2012) found out in late cropped cultivar ´Wojtek´the highest level of total polyphenols at the stage of late ripening fruit. Similarly, the highest content of flavonoid displayed LKL-3, LKL-14, LKL-33 and LKL-96 at the stage of ripened fruit. Otherwise, in the case of LKL-19 and LKL- 103 the content of flavonoids decreased in proportion to process of fruit maturation. Antioxidant activity of fruit was determined as the highest at the stage of ripened fruit for direct consumption except for LKL-96 and LKL-33 with the maximum value at the stage of overriped fruit. Similarly,

according to results of cluster analyses JURÍKOVÁ *et al.* (2014) clones of LKL-96 and LKL-103 differed from the rest of 15 evaluated clones in respect of antioxidant activity and content of anthocyanins achieved the highest values. In our reasearch the mentioned clones showed comparable content of total polyphenols, flavonoids and antioxidant activity. Otherwise, the earlier ripening clones LKL-3 and LKL-14, LKL-33 reached up higher values of antioxidant activity.

SKUPIEŃ *et al.* (2009) reported that the late ripening berries of the 'Czarna' cultivar and seedling 'N' showed an enhanced level of soluble solids and total polyphenols in comparison with early ripened cultivars. In our study the highest content of polyphenols in LKL-96 was observed at the beggining of the process of ripening than the values felt down.

In respect of the total polyphenols content (TPC), the content of flavonoids (FC) and the antioxidant capacity the most valuable clones at the stage of ripe fruit are following: LKL-14 (8.77; 2.57 g/kg FW; 9.07 g/kg), LKL-33 (6.77; 2.84 g/kg FW; 9.16 g/kg) and LKL-3 (8.19; 2.61 g/kg FW; 8.94 g/kg). Total polyphenols content of clone 38 (7.75 g/kg) determined by WOJDYLO *et al.* (2013) is similar to our assayed clone LKL-33.

JURÍKOVÁ *et al.* (2014) used the selected Russian cultivars of *Lonicera kamtschatica* (Sevast.) Pojark cultivated in conditions of the Czech Republic and found that the content of total polyphenols was cultivar dependent. SOCHOR *et al.* (2014) analysed 19 Russian cultivars of edible honeysuckle in the same conditions. The ´Goluboe vreteno´ was confirmed as the most valuable in total polyphenols content (8.658±109 g/kg) that is comparable with LKL-14, ´Zolushka´ had total phenol content (86.5 mg/GAE/kg) that is similar to LKL-3. Similarly, ROP *et al.* (2011) estimated the highest level of polyphenols in ´Zolushka´ 9.03 g/kg that represented higher value in comparison with our assayed clones. The fruit of *Lonicera caerulea* has 1.4 g/kg of flavonoids (PLEKHANOVA, 1990) that is comparable with LKL-96 and LKL-103. In the research work of KACZMARSKA *et al.* (2015) the content of flavonoids in 10 Polish cultivars of *Lonicera kamtschatica* (Sevast. Pojark.) ranged from 30.5-34.4 mg/100 g that represented higher values in comparison with assayed clones. In the same way, SKUPIEN *et al*. (2009) in genotype ´Czarna´ and seedling N, OCHMIAN *et al*., (2012) in cultivars ´Czerna´ and ´Wojtek´ found higher level of flavonoids. Otherwise, antioxidant activity of Polish genotypes ranged from 20.9 to 42.4%, in our research were measured a higher values among all the assayed clones. In the research work ROP *et al.* (2011) determined higher content of flavonoid among 12 cultivars of *Lonicera kamtschatica* (Sevast.) Pojark cultivated in the Czech Republic. MATUŠKOVIČ *et al.* (2009) evaluated antioxidant activity in relation to content of ascorbic acid and anthocyanins in the collection of 22 Klčov´s clones of *Lonicera kamtschatica*. The authors summed up that the LKL-19 in respect of assayed bioactive componds seem to be the most perspective clone. On the contrary, our results of reasearch pointed to the lowest value of total polyphenols, flavonoids and antioxidant activity in assayed 6 clones.

In the research study of JURÍKOVÁ *et al.* (2014) *Lonicera kamtschatica*-'Gerda 25' displayed the highest values of antioxidant activity (98.8 %) among assayed Russian cultivars cultivated in Zabcice in Czech Republic. The similar value of antioxidant activity was evaluated also in our research in case of LKL-33-overiped fruit stage of maturity (93.2%). PAULOVICSOVA *et al*. (2009) evaluated the antioxidant activity of lesser known fruit species cultivated in Botanical Garden of Slovak University of Agriculture in Nitra. Assayed values of

antioxidant activity of determined species are comparable with our clones in this way: LKL -3 with black mulberries (81.9 %) and LKL-14 with cornelian cherries (88.9 %). The antioxidant activity of LKL-33 is comparable with the cultivar ´Tomichka´ (8.77 g of ascorbic acid /kg fresh mass), LKL-14 with ´Kamchadalka´ 8.77 g of ascorbic acid /kg fresh mass determined by ROP *et al.* (2011). The antioxidant activity of selected 30 genotypes of *Lonicera kamtschatica* (Sevast.) Pojark determined by KUCHARSKA *et al.* (2017) (´Atut´ 8.80±0.17-´Jolanta´ 27.30±0.94) μmol TE/g fw represented lower values in comparison with our assayed clones.

The our detected values of antioxidant activity of *Lonicera kamtschatica* clones are in accord with with value of antioxidants of berries including blueberry, elderberry and black currant evaluated by JAKOBEK *et al.* (2007) and can be considered as comparative to raspberry and blackberry (MECH-NOWAK, 2014).

CONCLUSION

The results obtained in our research are useful for the selection of perspective clones of *Lonicera kamtschatica* (Sevast.) in respect for further utilisation in food industry. Furthemore, results indicated that optimal term for the harvesting of fruit at the stage of fully ripened fruit except for LKL-96 and LKL-103 totally differed from the assayed clones by content of flavonoids, polyphenols, antioxidant activity and later term of fruit maturity. The most promising among all analyzed clones LKL-14 and LKL-33, which were characterized by the high content of polyphenols, flavonoids as well as a high level of antioxidant activity. Statistical analyses proved significant differences in antioxidant activity and flavonoid content of selected 6 clones of *Lonicera kamtschatica* (Sevast.) Pojark.

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POVEZANOST FAZE ZRELOSTI VOĆA SA SADRŽAJEM POLIFENOLA, FLAVONOIDA I ANTIOKSIDATIVNE AKTIVNOSTI ODABRANIH KLONOVA *Lonicera kamtschatica* (Sevast.) Pojark

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Izvod

Ovaj rad se bavi procenom četiri faze zrelosti plodova 6 odabranih klonova (LKL-3, LKL-14, LKL-19, LKL-33, LKL-96 i LKL-103) Lonicera kamtschatica (Sevast.) Pojark u odnosu na sadržaj ukupnih polifenola, flavonoida i antioksidativne aktivnosti bobica. Eksperiment je postavljen u 5 ponavljanja za svaki klon u uslovima Botaničke bašte u Njitri (Slovačka Republika) 2016. godine. Rezultati analiza pokazali su da su u pogledu ukupnog sadržaja polifenola (TPC), sadržaja flavonoida (FC) i antioksidativne aktivnosti najznačajniji klonovi u fazi sazrevanja ploda sledeći: LKL-14 (8,77; 2,57 g / kg FV; 9,07 g / kg), LKL-33 (6,77; 2,84 g / kg FV; 9,16 g / kg) i LKL -3 (8,19; 2,61 g / kg FV; 8,94 g / kg). Rezultati statističkih analiza jednfaktorijalnom analizom varijanse pokazali su značajne razlike među ispitivanim klonovima u ukupnim flavonoidima i antioksidativnoj aktivnosti voća, ali ne i razlike u sadržaju ukupnih polifenola i ispitivanim fazama zrelosti ploda.

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