

## Sweet Cherry Pomological and Biochemical Characteristics Influenced by Rootstock

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Fruits of Lapins sweet cherry (*Prunus avium* L.) from 12-year-old trees on different rootstocks (F 12/1, Maxma 14, Piku 1, Edabriz, Gisela 5, Gisela 195/20, Weiroot 13, Weiroot 158, and Weiroot 72) were analyzed for pomological (fruit weight, % flesh of fruit weight, soluble solids content, titratable acidity, and firmness) and biochemical parameters (individual sugars, organic acids, and phenolic compounds) considering yield. For the first time, two procyanidins have been identified in sweet cherries using HPLC/MS: procyanidin B2 and procyanidin dimer ( $\lambda_{\max} = 275$  nm;  $[M - H]^-$  at  $m/z$  577 and MS<sup>2</sup> fragments at  $m/z$  425, 407, and 289). There were no significant differences between rootstocks in average yield per tree, except for Piku 1 (19.7 kg) with significantly the highest and Gisela 5 with significantly the lowest average yields per tree (7.7 kg). Significant differences in the measured parameters were ascertained among fruits of Lapins derived from different rootstock. Growing Lapins on Weiroot 72 and Edabriz resulted in high soluble solids content and fruit firmness. The lowest fruit firmness was measured on fruits from Weiroot 13, F 12/1, Gisela 195/20, and Maxma 14 trees. Fruits from Gisela 5 contained the lowest concentrations of glucose, fructose, sorbitol, sucrose, and citric acid, while fruits from F 12/1 contained the highest values of glucose, fructose, and shikimic acid. The content of phenolic compounds was the highest in fruits from Weiroot 72 tree, but the highest concentrations of procyanidin B2 and procyanidin dimer were ascertained in fruits from the Edabriz tree. Fruits from Weiroot 72 contained significantly higher concentration of phenols, bioactive compounds, compared to that in fruits from F 12/1. The content of individual and total anthocyanins did not differ significantly among rootstocks.

**KEYWORDS:** Cherries; *Prunus avium*; sugars; organic acids; phenolics; anthocyanins; yield; fruit weight; firmness; soluble solids; titratable acidity

### INTRODUCTION

Use of dwarfing rootstocks has dramatically increased the profitability of fruit production (1). Cherry rootstocks to impart a complete range of tree vigor are available now and are useful for different pedo-climatic conditions and cultivation systems (2). Cherry rootstocks have effects on grafted cultivars such as controlling tree size and yield efficiency (3–5), mineral composition of shoots (6), cessation of terminal shoot growth (1), variation in xylem structure (7), sweet cherry powdery mildew foliar infections (8), or response to drip irrigation (9).

Furthermore, different groups (10–13) have reported the effect of rootstock on fruit quality. Peach rootstocks influenced qualitative and nutritional characteristics of the fruit (11). Rootstocks of similar vigor produced peach fruits with different nutritional characteristics, indicating that the rootstock effect is more complex than just vigor (12). At harvest, soluble solids content and the soluble solids content/titratable acidity ratio of plums were not affected by rootstock (13).

High fruit quality is the goal of sweet cherry growers who target maximum economic result (14). Fruit quality is determined by pomological and biochemical characteristics, mainly dependent on the cultivar genotype, although the different size-controlling rootstocks also had a significant effect (15). Visual sweet cherry fruit characteristics, such as fruit size and skin color, influence consumer acceptance (16), the sugar/acid ratio has impacts on the perception of sweetness (17) and flavor (16), and, finally, phenolic antioxidants have various positive effects on human health (18–22).

Cherry rootstock evaluation has been very intensive over the past decade (2), mainly focused on plant adaptability, canopy volume, yield efficiency, and fruit weight. On the basis of available literature, there are no data about the effect of rootstocks on sweet cherry biochemical characteristics. Available are only the results of Gonçalves et al. (15) who studied the effect of rootstocks (Edabriz, Gisela 5, Maxma 14, CAB 11E, and *Prunus avium*) on physicochemical characteristics (fruit weight, firmness, soluble solids, titratable acidity, and color) of Burlat, Summit, and Van.

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The objective of our work was to quantify the influence of nine rootstocks on Lapins yield and several fruit quality parameters. We decided to include the data of one season because significant influence of climatic conditions in the specific year on the levels of phenolic acids was ascertained in cherries (23) and on plum quality parameters (color, fruit and stone weight, hardness, soluble solids content, and acidity) (24).

## MATERIALS AND METHODS

**Plant Material and Experimental Design.** Sweet cherry fruits were collected in 2008 from the 12-year-old experimental orchard of Fruit Growing Centre Bilje (Slovenia). Sweet cherry cultivar Lapins was grafted on 9 rootstocks of different vigor and genetic origin: F 12/1, Maxma 14, Piku 1, Edabriz, Gisela 5, Gisela 195/20, Weiroot 13 (W 13), Weiroot 158 (W 158), and Weiroot 72 (W 72) (Table 1), five trees per rootstock. The planting distance was 5 × 4 m. Trees were trained to the Spindle training system. Irrigation was applied with sprinklers.

**Picking and Measured Pomological Parameters.** Cherry fruits were picked on the basis of fruit color (bright red color; color chart, 3; Ctifl, Paris). Cherry fruits from all trees were picked all at once. The year 2008 was warmer comparable to a long-term average. Higher temperatures influenced equal ripening of Lapins fruit on different rootstocks. Yield per tree was measured. The variables (fruit weight, stone weight, firmness, soluble solids (SS) content, titratable acidity, and firmness) were measured immediately after picking. Ten fruits from each tree were weighed (fruit and stone), and 20 fruits from each tree were used for additional measurements: firmness (2 mm needle, penetrometer Chatillon DFGS50), soluble solid content (SSC) (digital refractometer ATAGO WM-7), and titratable acidity (TA) (Titrimo 719 S, Metrohm). On the basis of the measured data, the soluble solid content/titratable acidity ratio (SSC/TA) was calculated.

**Extraction and Determination of Sugars and Organic Acids.** The samples (0.5 kg) were packed in plastic bags, frozen, and kept at -20 °C until extraction. Different compounds (sugars, organic acids, and phenols) were analyzed from the whole edible part of fruit. Fruit samples were analyzed for the content of individual sugars (glucose, fructose, sucrose, and sorbitol) and organic acids (malic, citric, shikimic, and fumaric). The fruits were stoned and homogenized with a manual blender (Braun). Mashed (10 g) fruit was dissolved with 50 mL of twice distilled water for

30 min at room temperature. The extracted sample was centrifuged at 10,000g for 7 min at 10 °C (Eppendorf 5810 R centrifuge, Hamburg, Germany). The supernatant was filtered through a 0.45 μm cellulose ester filter (Macherey-Nagel), transferred into a vial, and used for analyses.

Analysis of sugars was performed using Thermo separation products HPLC with refractive index (RI) detector (Thermo Scientific, Waltham, MA). Separation of sugars was carried out using a Rezex RCM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, CA) with the column temperature maintained at 65 °C. The samples were eluted according to the isocratic method (25).

Organic acids were analyzed with HPLC, using a Rezex ROA column (300 × 7.8 mm; Phenomenex, USA) and a UV detector set at 210 nm (25).

The sugars and organic acids in cherry extracts were identified by their retention time characteristics. Concentrations were expressed as g per kg of fresh weight (FW) and fumaric and shikimic acids as mg per kg FW.

**Extraction and HPLC Analysis of Phenolic Compounds and Anthocyanins.** Samples were prepared according to the modified method (26): 5 g of sample was extracted with 10 mL of MeOH containing 3% formic acid and 1% 2,6-di-*tert*-butyl-4-methylphenol (BHT) using an ultrasonic bath. HPLC analysis was performed using a Surveyor system with a diode array detector (DAD), controlled by CromQuest 4.0 software (Thermo Finnigan, San Jose, CA). The anthocyanins were analyzed at 530 nm and other phenolics at 280 and 350 nm (rutin). The column used was a Gemini C<sub>18</sub> (150 × 4.6 mm 3 μm; Phenomenex) operated at 25 °C.

The elution solvents were 1% aqueous formic acid and 100% acetonitrile. The samples were eluted according to the gradient described in ref 27. The injection amount was 20 μL, and the flow rate was 1 mL/min. The anthocyanins and other phenolics were identified with a HPLC-Finnigan MS detector and an LCQ Deca XP MAX instrument. The quantities of neochlorogenic acid were calculated as equivalents of chlorogenic acid. *p*-Coumaroylquinic acid was calculated as equivalents of *p*-coumaric acid and the procyanidin dimer as equivalents of procyanidin B2. Concentrations were expressed as mg per 100 g FW. The quantities of cyanidin-3-rutinoside, pelargonidin-3-rutinoside, and peonidin-3-rutinoside were assessed from peak areas and calculated as equivalents of cyanidin-3-glucoside (CGE). The content of total anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents CGE/100 g of fresh cherry.

**Statistical Analysis.** Statistical analysis was conducted with the program Statgraphics Plus 4.0 (Statgraphics, Herndon, VA). One-way analysis of variance was used for analysis of the effect of rootstocks on the measured parameters. Differences between cultivars were estimated with Duncan's test ( $p < 0.05$ ). Correlation was performed between fruit weight and yield at  $p \leq 0.05$ . Multivariate statistical analysis (hierarchical cluster analysis, discriminate analysis, and classification), based on Ward's method using a square Euclidian distance, was conducted in order to interpret the differences in phenol content in Lapins fruit among different rootstocks.

## RESULTS AND DISCUSSION

**Yield.** The average yield of Lapins (Table 2) ranged from 7.7 (Gisela 5) to 19.7 kg/tree (Piku 1). Statistically, the average yield per tree was similar regarding rootstocks except for Gisela 5, when compared to that of W 13 and Piku 1. The results of the

**Table 1.** Rootstocks Used and Their Genetic Origin

rootstock	genetic origin
F 12/1	<i>Prunus avium</i> L.
Maxma 14	<i>Prunus mahaleb</i> L. × <i>Prunus avium</i> L.
Weiroot 13	<i>Prunus cerasus</i> L.
Piku 1	<i>Prunus avium</i> L. × ( <i>Prunus canescens</i> Bois. × <i>Prunus tomentosa</i> Thunb.)
Gisela 195/20	<i>Prunus canescens</i> Bois. × <i>Prunus cerasus</i> L.
Weiroot 158	<i>Prunus cerasus</i> L.
Gisela 5	<i>Prunus cerasus</i> L. × <i>Prunus canescens</i> Bois.
Edabriz	<i>Prunus cerasus</i> L.
Weiroot 72	<i>Prunus cerasus</i> L.

**Table 2.** Average Yield (kg/Tree), Fruit Weight (g), % Flesh of Fruit Weight, Firmness (N), Soluble Solid Content (SSC; °Brix Brix), Titratable Acidity (TA; % of Malic Acid), and SSC/TA Ratio of Lapins on Different Rootstocks<sup>a</sup>

	yield	fruit weight	% flesh of fruit	firmness	SSC	TA	SSC/TA
Edabriz	12.7 ± 1.4 abc	8.2 ± 0.2 ab	91.7 ± 0.7 ab	5.2 ± 0.1 bc	14.7 ± 0.5 a	0.39 ± 0.01 bc	37.5 ± 2.3 ab
F 12/1	12.2 ± 4.0 abc	8.4 ± 0.2 ab	92.7 ± 0.4 a	4.7 ± 0.1 de	13.4 ± 0.1 ab	0.37 ± 0.01 c	35.9 ± 0.9 abc
Gisela 195/20	13.5 ± 3.4 abc	7.1 ± 0.7 b	89.4 ± 0.8 b	4.8 ± 0.2 cde	12.9 ± 0.3 b	0.39 ± 0.02 bc	33.1 ± 1.3 bc
Gisela 5	7.7 ± 1.2 c	8.7 ± 0.5 a	91.8 ± 0.7 a	5.1 ± 0.2 bcd	13.9 ± 0.4 ab	0.36 ± 0.02 c	39.2 ± 3.3 a
Maxma 14	14.0 ± 4.2 abc	8.2 ± 0.3 ab	92.1 ± 0.8 a	5.0 ± 0.1 cde	14.0 ± 0.4 ab	0.45 ± 0.01 ab	31.5 ± 1.2 c
Piku 1	19.7 ± 1.4 a	7.7 ± 0.5 ab	91.5 ± 0.6 ab	5.4 ± 0.1 ab	13.9 ± 0.4 ab	0.41 ± 0.01 abc	34.2 ± 0.7 abc
W 13	18.0 ± 3.3 ab	8.7 ± 0.4 ab	91.0 ± 0.4 ab	4.6 ± 0.2 e	13.7 ± 0.8 ab	0.45 ± 0.03 ab	30.9 ± 1.3 c
W 158	15.5 ± 2.0 abc	7.7 ± 0.3 ab	91.4 ± 0.8 ab	5.2 ± 0.1 abc	13.6 ± 0.4 ab	0.44 ± 0.02 ab	31.5 ± 2.9 c
W 72	10.3 ± 1.3 bc	8.9 ± 0.3 a	90.9 ± 0.9 ab	5.7 ± 0.1 a	14.7 ± 0.4 a	0.47 ± 0.03 a	32.1 ± 2.7 bc

<sup>a</sup> Average values ± standard error are presented. Different letters in columns indicate significantly different values at  $p < 0.05$ .

**Table 3.** Mean Sugars Content (g/kg FW) with SE of Lapins Sweet Cherry on Different Rootstocks<sup>a</sup>

	glucose	fructose	sorbitol	sucrose	sum sugars
Edabriz	102.17 ± 27.03 abc	46.06 ± 8.48 bc	13.45 ± 3.11 b	7.75 ± 0.01 b	169.43 ± 34.81 bc
F 12/1	159.87 ± 16.95 a	69.88 ± 7.28 a	14.04 ± 0.29 b	7.77 ± 0.02 ab	251.56 ± 14.28 a
Gisela 195/20	72.19 ± 15.84 c	41.65 ± 5.55 c	9.55 ± 1.27 b	7.76 ± 0.01 ab	131.15 ± 22.52 c
Gisela 5	50.97 ± 11.94 c	35.76 ± 3.74 c	7.66 ± 1.25 b	7.74 ± 0.00 b	102.13 ± 16.84 c
Maxma 14	103.50 ± 20.32 abc	49.39 ± 6.38 bc	11.73 ± 1.72 b	7.77 ± 0.01 ab	172.40 ± 28.34 abc
Piku 1	86.18 ± 21.16 bc	45.57 ± 6.38 bc	12.38 ± 2.79 b	7.76 ± 0.01 ab	151.88 ± 29.37 c
W 13	148.12 ± 16.71 ab	63.11 ± 5.61 ab	21.29 ± 5.86 a	7.78 ± 0.01 a	240.03 ± 17.74 ab
W 158	96.43 ± 15.30 abc	49.66 ± 3.46 bc	11.61 ± 1.23 b	7.76 ± 0.01 ab	165.48 ± 19.91 bc
W 72	103.42 ± 16.58 abc	52.96 ± 4.79 abc	12.77 ± 1.83 b	7.77 ± 0.02 ab	176.92 ± 22.83 abc

<sup>a</sup> Different letters in columns indicate significantly different values at  $p < 0.05$ .

previous study (from the same orchard) show a significant reduction of tree volume and a significant increase of yield efficiency obtained with Gisela 5 compared to those of F 12/1 and Maxma 14 (4). Canopy volume of the rootstocks was vigorous (F 12/1, W 13, and Maxma 14), semivigorous (Piku 1), semidwarfing (Gisela 195/20, W 158, and Gisela 5), and dwarfing (Edabriz and W 72) (4).

**Pomological Characteristics.** Average fruit weight (Table 2) ranged from 8.9 to 7.1 g. Statistically, the highest fruit weight of Lapins was measured on rootstocks W 72 and Gisela 5; there were no differences between them and other rootstocks, except for Gisela 195/20 with significantly the lowest fruit weight (7.1 g). Fruit weight, beside cultivar genotype, depends on crop load (15) and fruit maturity stage (28–30). Lapins was picked earlier compared to the recommendations (29) but at the best maturity stage for specific weather conditions. Harvest decisions represent a compromise between delaying harvest to attain improved pomological characteristics versus picking early, before the cherries soften excessively (29). The most important parameter determining sweet cherry acceptability by consumers is bright red color (16). The earliness of the harvest is demonstrated also with the lower average fruit weight than optimal (29). In our study, crop load had a significant effect on the average fruit weight of Lapins fruit only from trees on Gisela 5 ( $r = -0.93$ ). The effect of different rootstocks on fruit size was variable in sweet cherry (15) depending on cultivar but in the case of plums because of strong interaction of the rootstock and year (24). From the data of stone weight, percentage to fruit weight was calculated. Fruits of Lapins on Gisela 195/20 had significantly higher percentage of stone in the fruit weight compared to that of F 12/1, Gisela 5, and Maxma 14.

Soluble solid content (SSC) varied from 12.9 (Gisela 195/20) to 14.7°Brix (Edabriz and W 72) and is lower than data measured by Drake and Elfving (29) and Dever et al. (31) because the maturity stage. Lapins from trees on Edabriz and W 72 had significantly higher SSC compared to that of Gisela 195/20. Harvesting Lapins up to 5 days later than normal commercial harvest resulted in increases in SSC (29). Van cherries had the highest SSC when grafted on dwarfing rootstocks Edabriz and Gisela 5 (15). Peach from the less vigorous trees had the highest SSC (11). It has been shown that dwarf rootstocks are generally able to send more nutrients to the fruit because there is less competition from the vegetative parts (32).

Differences in average titratable acidity (TA) were ascertained in fruit of Lapins on different rootstocks. The highest acidity was measured in fruit from the W 72 rootstock (0.47%), being significantly different from that of fruit from trees on rootstocks Edabriz and Gisela 195/20 (0.39%), F 12/1 (0.37%), and Gisela 5 (0.36%). TA of Lapins varied from 0.43 to 0.58% during 3 years and 3 ripening stages (29). Among 11 cultivars and maturity stages, the values of TA ranged from 0.7 to 1.5 g 100 g<sup>-1</sup> (30), while for cultivars Brooks and Bing, Crisosto et al. (16) reported

from 0.5 to 1.0%. An accumulation of acidity was observed in 11 sweet cherry cultivars as the harvesting date was delayed (28), but Drake and Elfving (29) reported influence of harvest date on TA of Lapins only in 1 of the 3 years.

Fruit of Lapins from the rootstock Gisela 5 had significantly higher SSC/TA ratio compared to that of Gisela 195/20, W 158, Maxma 14, W 13, and W 72, the last three rootstocks having the lowest values. A high SSC/TA ratio means high soluble solid content by low acidity. With regard to the fact that SSC/TA ratio has a significant impact on the perception of sweetness (17), it can be assumed that the taste of Lapins fruits from Gisela 5 trees was more sweet compared to that of fruits from Maxma 14, W 13, and W 158 trees. For sweet cherries, higher SSC/TA ratios correlated with higher eating quality (16). Different peach and plum rootstocks did not affect SSC/TA ratios (11, 13).

Fruit firmness varied from 4.6 to 5.7 (Table 2) and is lower than data measured by Drake and Elfving (29). Fruit firmness decreased sharply as fruit weight increased (28). Statistically, the fruit of Lapins was more firm on rootstock W 72 than on Edabriz, F 12/1, Gisela 195/20, Gisela 5, Maxma 14, and W 13. It is interesting, that the lowest value of firmness was ascertained on vigorous rootstock W 13, compared to that of W 72, W 158, Edabriz, Gisela 5, and Piku 1. Gonçalves et al. (15) observed a distinct effect of dwarfing and invigorating rootstocks only in Van cherries: the highest firmness when grafted on dwarfing rootstocks. The fruit of Suncrest peach was not significantly affected by rootstock (11), but in plums (13, 24), an effect of rootstock on fruit firmness was ascertained.

**Sugar and Organic Acid Content.** There were significant differences between rootstocks in the concentration of glucose, fructose, sorbitol, sucrose, and the sum of sugars in the fruit of Lapins (Table 3), which were similar in concentrations to data of the previous study (33). Monosaccharides are predominant in sweet cherry fruits. Statistically, the highest content of glucose was measured in fruits from rootstock F 12/1, which was different from that of Gisela 195/20, Gisela 5, and Piku 1. F 12/1 resulted in fruits with higher fructose content compared to that of Edabriz, Gisela 195/20, Gisela 5, Maxma 14, Piku 1, and W 158. The lowest concentrations of sorbitol and sucrose were ascertained in Lapins fruits from the Gisela 5 rootstock, which was different only from W 13. Fruits of Lapins had the highest sum of sugars on F 12/1 and the lowest on Gisela 5 rootstock. Fruits of Lapins on rootstock F 12/1 regarding the sum of sugars were significantly different from those on rootstocks Edabriz, W 158, Piku 1, Gisela 195/20, and Gisela 5.

The content of organic acids in the fruit of Lapins is shown in Table 4. The content of malic acid in fruits of Lapins varied from 2.90 to 8.18 g/kg FW, citric acid from 0.22 to 0.93 g/kg FW, shikimic acid from 2.56 to 8.17 mg/kg FW, and fumaric acid from 0.35 to 1.97 mg/kg FW. Similar content of acids were measured in our previous study (33). Statistically, the highest content of malic

**Table 4.** Mean Organic Acids Content with SE of Lapins Sweet Cherry on Different Rootstocks<sup>a</sup>

	(g/kg FW)		(mg/kg FW)		sum acids (g/kg FW)
	malic acid	citric acid	shikimic acid	fumaric acid	
Edabriz	4.17 ± 0.98 bc	0.42 ± 0.14 b	5.29 ± 1.08 ab	0.87 ± 0.14 bc	4.59 ± 1.11 bc
F 12/1	6.96 ± 1.56 ab	0.60 ± 0.16 ab	8.17 ± 2.09 a	1.39 ± 0.37 ab	7.57 ± 1.69 ab
Gisela 195/20	2.90 ± 0.55 c	0.38 ± 0.12 b	2.56 ± 1.02 b	0.35 ± 0.14 c	3.28 ± 0.57 c
Gisela 5	4.39 ± 1.04 bc	0.22 ± 0.05 b	3.55 ± 1.03 b	0.71 ± 0.16 bc	4.61 ± 1.07 bc
Maxma 14	3.67 ± 0.81 c	0.30 ± 0.07 b	3.58 ± 0.87 b	0.65 ± 0.21 bc	3.98 ± 0.73 c
Piku 1	3.05 ± 0.89 c	0.28 ± 0.06 b	4.95 ± 1.01 ab	0.88 ± 0.29 bc	3.33 ± 0.89 c
W 13	8.18 ± 1.57 a	0.93 ± 0.24 a	7.89 ± 1.11 a	1.97 ± 0.58 a	9.12 ± 1.81 a
W 158	4.71 ± 0.58 bc	0.38 ± 0.07 b	5.98 ± 0.91 ab	1.06 ± 1.13 bc	5.09 ± 0.65 bc
W 72	3.39 ± 0.60 c	0.22 ± 0.04 b	3.70 ± 1.08 b	0.66 ± 0.20 bc	3.61 ± 0.60 c

<sup>a</sup> Different letters in columns indicate significantly different values at  $p < 0.05$ .

**Table 5.** Phenolics Content of Lapins Sweet Cherry on Different Rootstocks (mg/100 g FW)<sup>a</sup>

	neochlorogenic acid	<i>p</i> -poumaroylquinic acid	chlorogenic acid	rutin
Edabriz	26.49 ± 1.69 abc	3.19 ± 0.26 a	5.67 ± 0.62 ab	1.10 ± 0.19 a
F 12/1	21.56 ± 3.04 c	2.58 ± 0.22 a	4.34 ± 0.68 b	0.94 ± 0.11 a
Gisela 195/20	26.44 ± 1.50 abc	2.91 ± 0.22 a	5.55 ± 0.12 ab	1.11 ± 0.13 a
Gisela 5	28.06 ± 2.67 abc	2.95 ± 0.32 a	5.96 ± 0.46 a	0.90 ± 0.16 a
Maxma 14	31.96 ± 1.02 ab	3.36 ± 0.17 a	5.88 ± 0.18 a	0.99 ± 0.06 a
Piku 1	26.72 ± 1.35 abc	2.59 ± 0.33 a	5.15 ± 0.11 ab	0.89 ± 0.12 a
W 13	25.50 ± 1.79 bc	3.06 ± 0.13 a	5.38 ± 0.32 ab	0.93 ± 0.06 a
W 158	29.49 ± 3.02 ab	3.22 ± 0.44 a	5.26 ± 0.40 ab	0.93 ± 0.08 a
W 72	33.76 ± 2.66 a	3.46 ± 0.31 a	6.23 ± 0.49 a	1.21 ± 0.16 a

<sup>a</sup> Average values ± standard error are presented. Different letters in columns indicate significantly different values at  $p < 0.05$ .

**Table 6.** Phenolics Content of Lapins Sweet Cherry on Different Rootstocks (mg/100 g FW)<sup>a</sup>

	catechin	epicatechin	procyanidin B2	procyanidin dimer	sum phenols
Edabriz	2.43 ± 0.15 a	5.38 ± 0.25 ab	2.72 ± 0.38 a	4.43 ± 0.42 a	54.41 ± 5.05 ab
F 12/1	1.96 ± 0.23 ab	4.93 ± 0.38 b	1.87 ± 0.13 a	2.94 ± 0.45 b	41.12 ± 3.96 b
Gisela 195/20	2.11 ± 0.10 ab	5.46 ± 0.37 ab	2.26 ± 0.39 a	3.77 ± 0.23 ab	49.61 ± 1.89 ab
Gisela 5	2.22 ± 0.40 ab	5.38 ± 0.51 ab	2.23 ± 0.15 a	3.81 ± 0.34 ab	51.51 ± 7.79 ab
Maxma 14	2.25 ± 0.26 ab	6.24 ± 0.23 a	2.23 ± 0.11 a	4.30 ± 0.10 a	57.21 ± 6.66 ab
Piku 1	1.64 ± 0.26 b	5.41 ± 0.21 ab	1.92 ± 0.19 a	3.83 ± 0.14 ab	48.15 ± 6.10 ab
W 13	1.94 ± 0.11 ab	5.89 ± 0.55 ab	1.95 ± 0.64 a	4.07 ± 0.51 a	48.72 ± 3.04 ab
W 158	2.12 ± 0.19 ab	5.33 ± 0.20 ab	2.03 ± 0.11 a	3.80 ± 0.33 ab	52.18 ± 5.75 ab
W 72	2.54 ± 0.09 a	6.24 ± 0.40 a	2.11 ± 0.39 a	4.34 ± 0.29 a	59.89 ± 3.84 a

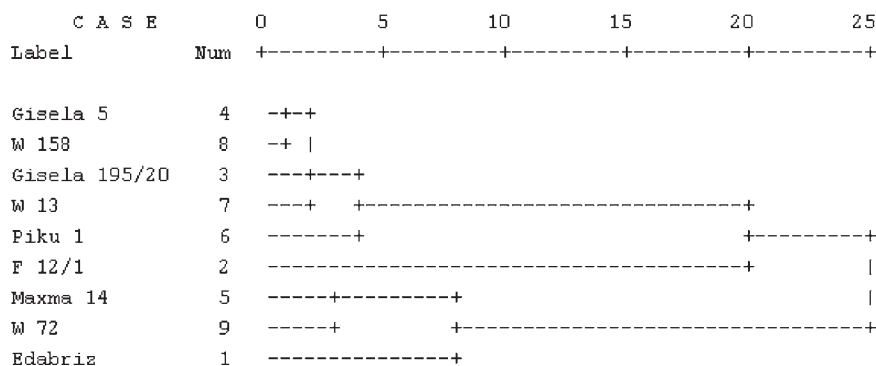
<sup>a</sup> Average values ± standard error are presented. Different letters in columns indicate significantly different values at  $p < 0.05$ .

acid and citric acid in Lapins fruits was measured on W 13 (Table 4). The lowest content of malic acid was ascertained on Gisela 195/20, being lower compared to that of F 12/1 and W 13. The lowest content of citric acid was measured on the rootstock of Gisela 5, which was significantly lower only from fruits on W 13. Differences were ascertained between rootstocks regarding shikimic and fumaric acid, minor organic acids in sweet cherry fruits, the lowest content on Gisela 195/20. Fruits from W 13 had the highest fumaric acid content. Fruits of Lapins had the highest sum of organic acids on W 13 (9.12 g/kg FW) and the lowest on Gisela 195/20 (3.28 g/kg FW) rootstock.

**Phenols.** The content of phenolic compounds, without anthocyanins, analyzed in Lapins fruit, is shown in Tables 5 and 6. Neochlorogenic acid, *p*-coumaroylquinic acid, chlorogenic acid, rutin, catechin, epicatechin, and two procyanidins (B2 and a not exactly assigned procyanidin dimer) were detected in the study. Concentrations of phenolics in the present study are higher compared to those in our previous study (33) because of the different method used and are still low (23), but the proportions among individual phenolics are similar. For the first time, two procyanidins have been identified in sweet cherries using HPLC/MS with different retention times: procyanidin B2 ( $R_t = 14.2$  min) and

procyanidin dimer ( $R_t = 9.6$  min). The procyanidin dimer could not be exactly assigned because of the lack of a standard. Spectral characteristics of both procyanidins are  $\gamma_{\max} = 275$  nm;  $[M - H]^-$  at  $m/z$  577; and  $MS^2$  fragments at  $m/z$  425, 407, and 289. The procyanidin dimer ranged from 2.94 to 4.43 mg/100 g FW and procyanidin B2 from 1.87 to 2.72 mg/100 g FW.

Differences in the concentrations of neochlorogenic acid, chlorogenic acid, epicatechin, procyanidin dimer, and the sum of phenols were ascertained in the study. The highest concentrations of phenolic compounds, except for procyanidins, and the sum of phenols were measured in Lapins fruits from W 72 trees, although not always significantly different from that of other rootstocks. The lowest concentrations of individual phenols, except for rutin and catechin, and the sum of phenols were measured in Lapins fruits from F 12/1 trees. The F 12/1 tree produced fruit with significantly lower concentration of phenolics compared to that of W 72. The levels of phenolic acids besides other factors depended on maturity stage (23, 30). Fruits from F 12/1 could be less ripe than W 72, but other results of the study did not support this statement. According to research of the beneficial effects of sweet cherries on human health (19–21) and results of multivariate analysis (Figure 1), it can be assumed that fruit from



**Figure 1.** Dendrogram of 9 cherry rootstocks using the Ward method based on square Euclidian distance from phenol content.

**Table 7.** Anthocyanin Composition and Sum of Lapins Sweet Cherry on Different Rootstocks Presented As mg CGE/100 g with SE<sup>a</sup>

	cyanidin 3-glucoside	cyanidin 3-rutinoside	pelargonidin 3-rutinoside	peonidin 3-rutinoside	sum anthocyanins
Edabriz	0.29 ± 0.05 a	10.10 ± 0.91 a	0.77 ± 0.05 ab	0.52 ± 0.01 b	11.68 ± 0.75 a
F 12/1	0.35 ± 0.07 a	9.78 ± 1.27 a	0.93 ± 0.19 a	1.11 ± 0.26 a	12.17 ± 6.2 a
Gisela 195/20	0.33 ± 0.09 a	7.47 ± 1.88 a	0.56 ± 0.14 b	0.65 ± 0.12 b	9.01 ± 2.23 a
Gisela 5	0.29 ± 0.02 a	7.56 ± 0.65 a	0.58 ± 0.04 b	0.60 ± 0.08 b	9.03 ± 0.78 a
Maxma 14	0.41 ± 0.07 a	9.45 ± 1.40 a	0.81 ± 0.04 ab	0.68 ± 0.10 b	11.35 ± 1.71 a
Piku 1	0.26 ± 0.05 a	7.39 ± 1.32 a	0.58 ± 0.10 b	0.57 ± 0.04 b	8.80 ± 1.49 a
W 13	0.31 ± 0.06 a	6.74 ± 0.87 a	0.53 ± 0.06 b	0.75 ± 0.18 b	8.33 ± 1.20 a
W 158	0.29 ± 0.04 a	7.93 ± 0.96 a	0.62 ± 0.08 ab	0.56 ± 0.04 b	9.4 ± 1.10 a
W 72	0.35 ± 0.06 a	8.56 ± 0.47 a	0.67 ± 0.02 ab	0.74 ± 0.17 b	10.32 ± 0.62 a

<sup>a</sup> Average values ± standard error are presented. Different letters in columns indicate significantly different values at  $p < 0.05$ .

W 72 and F 12/1 differed in the content of phenols/bioactive compounds.

The anthocyanins identified in Lapins fruit (**Table 7**) were the same as those identified in the previous study (33). Statistically similar concentrations of cyanidin 3-glucoside were measured in Lapins fruits, the highest being from trees on Maxma 14 and the lowest from trees on Piku 1. Although not statistically significant, the highest concentrations of cyanidin 3-rutinoside were obtained on Edabriz and the lowest on W 13. Fruits of Lapins on F 12/1 contained the highest concentrations of pelargonidin 3-rutinoside and peonidin 3-rutinoside. The total levels of anthocyanins are higher in ripe sweet cherries than in partially ripe ones (23, 30). It can be assumed that Lapins fruits have been picked in a similar maturity stage with regard to the fact that the sum of anthocyanins did not differ significantly among rootstocks, but the results could be different when harvest was delayed.

Similarity among rootstocks according to the phenol content in Lapins fruit has been researched with methods for cluster analysis. The dendrogram (**Figure 1**) subdivided rootstocks into three major groups: group 1 comprising semidwarfing rootstocks Gisela 5, W 158, and Gisela 195/20; vigorous rootstock W 13; and semivigorous rootstock Piku 1. It is interesting, that F 12/1 (*Prunus avium* L.) (group 2) shows the least similarity with other rootstocks. Dwarfing rootstocks W 72 and Edabriz were classified in group 3 with vigorous Maxma 14.

In conclusion, rootstocks affected the differences in Lapins pomological and biochemical characteristics. Measured parameters were rather low because of maturity stage. Fruit of Lapins on the F 12/1 tree had the lowest stone weight (the highest part of the flesh in the fruit weight), the highest concentrations of glucose, fructose, the sum of sugars, shikimic acid, pelargonidin 3-rutinoside, peonidin 3-rutinoside, and the sum of anthocyanins and the lowest concentrations of individual phenols (except for rutin and catechin) and the sum of phenols. Gisela 195/20 rootstock resulted in statistically the highest part of the stone in the Lapins fruit and the lowest fruit weight and content of soluble solids,

accompanied with low sugar and organic acid concentrations. Fruit of Lapins on the W 13 tree had significantly the lowest firmness, SSC/TA ratio, and the lowest concentrations of cyanidin 3-rutinoside, pelargonidin 3-rutinoside, and total anthocyanidins, and significantly the highest concentration of sorbitol, sucrose, malic acid, citric acid, fumaric acid, and the sum of organic acids. Using Edabriz resulted in high concentrations of procyanidin dimer and procyanidin B2 and soluble solids content in the fruits of Lapins. In our study, Lapins on W 72 rootstock gave fruits with the highest fruit quality based on fruit weight, firmness, soluble solids content, and concentration of phenols as health promoting substances.

Undoubtedly, plant adaptability, canopy volume, and yield efficiency are the most important criteria in sweet cherry rootstock testing. But impact on fruit quality is important as well. Fruit quality determines consumer acceptance, the content of nutrition attributes, and bioactive compounds as well.

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