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IN NOVI SAD**

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4th

International Congress

**Food Technology,
Quality and Safety**



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Preliminary programme

Committee invites PhD students to apply and orally present the results of their research within a congress session specially dedicated to young researchers. Best presentations will be rewarded.

23rd October

8:00-9:00	Registration at desk
9:00-10:00	Opening ceremony
10:00-10:30	Plenary lecture
10:30-11:00	Coffee break
11:00-13:00	Plenary lectures (30 min each)
13:00-14:00	Lunch
14:00-16:30	Round table Innovative trends in food & feed science
17:00-22:00	Social programme (sightseeing, exhibition and tasting of local and traditional food products)

24th October

8:00-8:30	Registration at desk
8:30-9:00	Plenary lecture
9:15-10:45	Three parallel sessions (in each one keynote lecture (20 min), 4 oral presentations (15 min), discussion (10 min))
10:45-11:15	Coffee break
11:15-12:45	Three parallel sessions (in each one keynote lecture (20 min), 4 oral presentations (15 min), discussion (10 min))
12:45-14:00	Lunch
14:00-14:30	Plenary lecture
14:45-16:15	Three parallel sessions (in each one keynote lecture (20 min), 4 oral presentations (15 min), discussion (10 min))
16:15-16:30	Coffee break
16:30-18:00	Three parallel sessions (in each one keynote lecture (20 min), 4 oral presentations (15 min), discussion (10 min))

25th October

8:00-8:30	Registration at desk
8:30-9:00	Plenary lecture

9:15-10:45	Two parallel sessions (in each one keynote lecture (20 min), 4 oral presentations (15 min), discussion (10 min))
10:45-11:15	Coffee break
11:15-12:45	Two parallel sessions (in each one keynote lecture (20 min), 4 oral presentations (15 min), discussion (10 min))
12:45-14:00	Lunch
14:00-14:30	Plenary lecture
14:45-16:15	Two parallel sessions (in each one keynote lecture (20 min), 4 oral presentations (15 min), discussion (10 min))
16:15-16:30	Coffee break
16:30-18:00	Closing ceremony (best poster award, reports from food expo, round table)
20:00	Gala dinner

FRUIT TRAITS, CAPSAICIN AND DIHYDROCAPSAICIN CONTENT IN SOME SERBIAN HOT PEPPERS

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ABSTRACT

The diversity of fruit traits and pungency level in hot pepper (*Capsicum annuum* L.) are very important for consumers. Therefore the aim of this study was to evaluate phenotypic fruit traits and content of capsaicin and dihydrocapsaicin in nine hot pepper genotypes (five varieties and four breeding lines) originating from Serbia. A field trial was conducted on chernozem soil in 2017 at the Institute of Field and Vegetable Crops (IFVCNS) in Novi Sad, Serbia. The experiment was established in a randomized block design with two replications and 20 plants per replicate. Six quantitative fruit traits (weight, length, diameter, index, pericarp thickness, and total soluble solids) were used for fruit characterization. Ten fruits in technological maturity were analysed per genotype, and dried. The sample of each dry pepper was ground and 1 g was prepared for capsaicin and dihydrocapsaicin analysis into GC-MS. The pungency levels were calculated in Scoville heat units.

The longest fruits (22.12 cm and 19.80 cm) had breeding lines 243/17 and 238/17 respectively, while the fruit length of other genotypes ranged from 6.15 to 15.83 cm. Variety Somborka had the highest pericarp thickness and fruit weight. Total soluble solids (TSS) in all genotypes varied between 4.12 - 8.16 °Brix, however, the highest TSS were noted in three genotypes which belong to small pepperoni fruit type. According to performed analysis, the lowest capsaicin and dihydrocapsaicin contents were measured in Somborka variety, while the new variety NS Vatrema had the highest dihydrocapsaicin content and pungency.

Keywords: *Capsicum*, *Capsaicin*, *Scoville Heat Unit*, *Serbia*

INTRODUCTION

Pepper fruits (*C. annuum* L.) are consumed as a fresh vegetable and processed in different ways, as well as for drying as spice. Pepper is one of the major vegetable species in Serbia. Comparing to other vegetable crops (excluding potato), pepper (sweet and hot) has the first rank in Serbia with 17,386 ha of total growing area in 2017 (Statistical Office of the Republic of Serbia, 2018). Habits of pepper consumers are specific for the different regions of Serbia and therefore the people consume pepper fruits with various shapes, sizes and colors (Danojevic *et al.*, 2016). However, hot pepper varieties are not so widely grown as sweet pepper cultivars. The market value of hot peppers depends mainly on pungency, fruit type, pericarp thickness, color and flavor.

Besides its use as a spice, hot peppers and their isolated constituents including capsaicinoids are appreciated because of their beneficial therapeutic effects, including antioxidants, anti-inflammatory, anticancer, antimicrobial and positive immunomodulatory effects (Popelka *et al.*, 2017).

The capsaicinoids are a specific class of compounds in chili pepper fruit that causes the spicy sensation (pungency). The main capsaicinoid is capsaicin, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. Of these compounds, capsaicin, and dihydrocapsaicin account for about 90% of capsaicinoids in chili pepper fruit (González-Zamora *et al.*, 2015).

Many local pepper populations were produced by spontaneous crossing and selection by pepper growers (Stevanović & Miladinović, 1989). For that reason, the former Yugoslavia was very rich in hot pepper germplasm (Zewdie & Zeven, 1997). Despite the fact of great number of local hot pepper genotypes in Serbia, there are no published papers about their pungency level. Therefore, the present study was undertaken to screen the pungency and

main fruit traits of hot pepper cultivars in Serbia and perspective breeding lines from the Institute of Field and Vegetable Crops.

MATERIAL AND METHODS

The trial was started in 2017 at the Institute of Field and Vegetable Crops (Novi Sad, Serbia) with 9 genotypes of hot peppers (5 varieties [Somborka, Feferona crvena, Feferona žuta Plamena, NS Vatrena] and 4 breeding lines [233/17, 238/17, 243/17, 244/17]). The seed was sown on March 28th, 2017 in a plastic greenhouse without heating. Plants were transplanted in an open field on June 8th, 2017. The planting distance was 70 cm between rows and 25 cm in the row. The experiment was established in a randomized block design with two replications and 20 plants per replicate (one row). The plants were irrigated with a drip-by-drip system. Pesticides have not been applied. The fruits were harvested on October 24th, 2017 in technological maturity. Following traits: Fruit Weight (g) [FW], Fruit Length (cm) [FL], Fruit Diameter (cm) [FD], Fruit Index - Fruit Length/Diameter [FI], Pericarp Thickness (mm) [PT], and Total Soluble Solids (°Brix) [TSS] were used for fruit characterisation. Ten fruits per genotype were analysed, after that the fruits were dried at 70°C to a constant weight and kept for further analysis.

HPLC-grade absolute ethanol (Carlo Erba) and HPLC-grade dichloromethane (Sigma-Aldrich) were used as solvents. Capsaicin (≥ 99.0%) and dihydrocapsaicin (≥ 97.0%) were obtained from Sigma-Aldrich, Switzerland.

The sample of each dry pepper was ground and 1 g of powder was weighted and placed in a Soxhlet apparatus with 150 ml of ethanol. The solution was refluxed for 5 h. After cooling, the solution was evaporated to dryness in a rotary evaporator system, dissolved with a mixture of CH₂Cl₂:EtOH (25:1, v/v), transferred to 5 ml volumetric flask and filled to the mark. Since the solution was not clear, it was centrifuged for 5 min at 10000 rpm. Afterward, it was transferred to the vial and injected into GC-MS. The GC-MS analysis was performed on an Agilent 6890N series gas chromatograph equipped with a CombiPal CTC Analytics autosampler, an HP5-MS column (30 m length, 250 μm internal diameter, film thickness 0.25 μm, Agilent J&W GC Columns, Agilent Technologies), and a single quadrupole mass spectrometer Agilent 5975B. The injector temperature was 230°C, with an injection volume of 1 μl in a splitless mode. Carrier gas (He) had a constant flow rate of 1.0 ml min⁻¹. The initial oven temperature was 40°C for 1 min, then programmed to 300°C at ramp rate of 3°C/min, final temperature hold for 5 min. The MS source and the transfer line temperatures were set to 230°C and 280°C. The mass range analysed by the mass spectrometer was 35.00 – 500.00 amu. In SIM mode ions with *m/z* 137, 152, 305 and 307 were monitored.

Capsaicinoid content was converted to Scoville Heat Unit (SHU) by multiplying the concentration of capsaicinoid in dry weight of pepper in parts per million (ppm). Scoville Heat Unit was determined according to formula

Total Scoville Heat Unit = [capsaicin (ppm) + dihydrocapsaicin (ppm)] × 16.1 (Gayathri et al., 2016). Software package Statistica ver. 13.2, (StatSoft. Inc. 2016) were used for Bonferroni test at *p* < 0.05, and graphs.

RESULTS AND DISCUSSION

Morphological analysis of the fruits, showed significant variability among tested varieties and lines (Table 1). The heaviest fruit weight had variety Somborka (65.04 g), followed by 244/17, 243/17, and Plamena (41.33 g, 38.67 g, and 38.52 g respectively). Varieties which belong to small pepperoni fruit type (Feferona crvena, NS Vatrena and Feferona žuta) had the lowest fruit weight (3.29 - 4.00 g). The highest pericarp thickness was observed in variety Somborka, while small pepperoni fruits had the lowest pericarp thickness (0.52-0.79 mm). Obtained data showed that plants with thicker pericarp had higher fruit weight. In addition, results of our previous research, indicated the positive correlation between fruit weight and pericarp thickness (Danojević *et al.*, 2016).

Breeding lines 243/17 and 238/17 had the longest fruits (22.12 cm and 19.80 cm respectively), while the fruit length of other genotypes ranged from 6.15 to 15.83 cm. Total soluble solids (TSS) in all genotypes varied between 4.12 - 8.16 °Brix. However, the highest TSS were noted in three genotypes which belong to small pepperoni fruit type. By the evaluation of some quantitative and qualitative fruit traits, small pepperoni genotypes were separated from other hot pepper varieties such as Plamena and Somborka (Danojevic *et al.*, 2017).

Table 1. Mean values of evaluated fruit traits in hot pepper genotypes

Genotype	Fruit Weight (g)	Fruit Length (cm)	Fruit Diameter (cm)	Fruit Index	Pericarp Thickness (mm)	Total Soluble Solids (°Brix)
Somborka	65.04 ^a	7.69 ^d	5.39 ^a	1.43 ^c	4.45 ^a	4.12 ^e
Plamena	38.52 ^b	14.25 ^b	2.68 ^b	5.36 ^b	2.56 ^b	5.78 ^{cd}
233/17	14.27 ^d	10.71 ^c	1.64 ^d	6.59 ^b	1.90 ^b	5.91 ^{bcd}
238/17	29.93 ^c	19.80 ^a	2.13 ^c	9.63 ^a	2.46 ^b	6.15 ^{bcd}
243/17	38.67 ^b	22.12 ^a	2.38 ^{bc}	9.52 ^a	2.07 ^b	4.82 ^{de}
244/17	41.33 ^b	15.83 ^b	2.76 ^b	5.79 ^b	2.41 ^b	5.42 ^{de}
NS Vatrema	3.63 ^e	6.15 ^d	1.04 ^e	5.98 ^b	0.52 ^c	7.10 ^{abc}
Feferona crvena	3.29 ^e	6.24 ^d	0.88 ^e	7.06 ^b	0.57 ^c	7.17 ^{ab}
Feferona žuta	4.00 ^e	6.39 ^d	0.98 ^e	6.52 ^b	0.79 ^c	8.16 ^a

Different letters show significant differences between genotypes ($P < 0.05$) according to the Bonferroni test.

According to performed analysis, Feferona žuta had the highest capsaicin content (5312 mg kg⁻¹ of dry matter), then NS Vatrema (4702 mg kg⁻¹ of dry matter) while the lowest capsaicin content was measured in Somborka, 244/17, Feferona crvena and Plamena (47-295.5 mg kg⁻¹ of dry matter) (Figure 1).

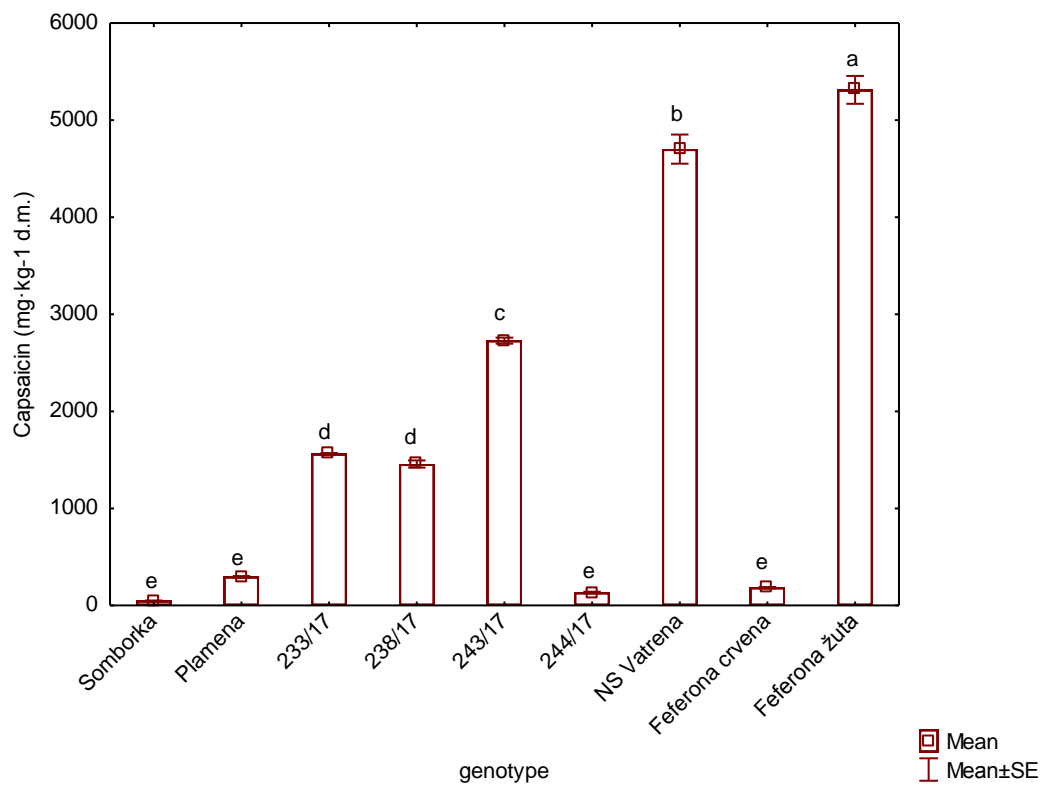


Figure 1. Capsaicin content in hot pepper genotypes. Different letters show significant differences between genotypes ($P < 0.05$) according to the Bonferroni test.

The obtained data showed a diversified content of dihydrocapsaicin among the genotypes. The highest dihydrocapsaicin content had a new variety NS Vatreña (4981 mg kg^{-1} of dry matter), while Feferona žuta had $2680.5 \text{ mg kg}^{-1}$ of dry matter, nearly two times lower than the firstly ranked (Figure 2). Somborka and breeding line 244/17 had the lowest dihydrocapsaicin content. Breeding lines: 233/17, 238/17, 243/17 had dihydrocapsaicin content between $1057\text{-}2075 \text{ mg kg}^{-1}$ of dry matter.

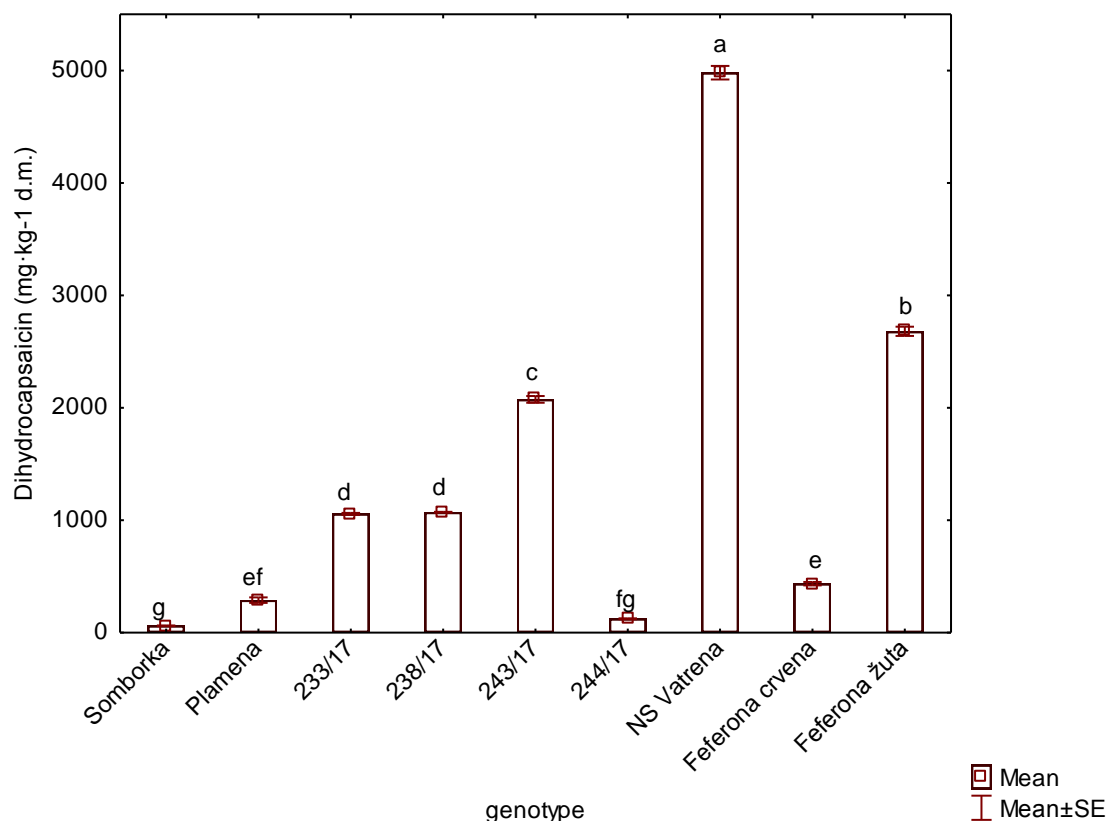


Figure 2. Dihydrocapsaicin content in hot pepper genotypes.

Different letters show significant differences between genotypes ($P < 0.05$) according to the Bonferroni test.

There are five levels of pungency classified using Scoville heat units (SHU): non-pungent (0–700 SHU), mildly pungent (700–3,000 SHU), moderately pungent (3,000–25,000 SHU), highly pungent (25,000–70,000 SHU) and very highly pungent (>80,000 SHU) (Weiss, 2002). According to this classification NS Vatreña and Feferona žuta, were very highly pungent (155896.3 and 128679.3 SHU, respectively) (Figure 3). Breeding line 243/17 with pungency of 77336 SHU was between highly and very highly pungent genotypes, while other two breeding lines 233/17 and 238/17 had pungency levels 42157 and 40725 SHU, respectively, and belong to highly pungent. Moderately pungent were Feferona crvena, Plamena and 244/17. The lowest SHU was noted in Somborka and this variety could be classified as mildly pungent.

Fruits of Somborka variety is widespread in Serbia and used fresh or with sour cream. Pepperoni type (Feferona crvena and Feferona žuta) were used usually as pickled peppers. The new variety NS Vatreña was selected also for this purpose. Although NS Vatreña (orange ripe fruit) originated as a mutation from Feferona crvena (red ripe fruit), it has more than fifteen times higher SHU than Feferona crvena. Orange fruits and higher pungency are the traits which separate NS Vatreña from other varieties of small pepperoni type. The breeding line 243/17 with relatively high fruit weight 38.67 g, longer fruits 22.12 cm, pericarp thickness higher than 2 mm and 77336 SHU could be a perspective material for further breeding or possible new hot pepper variety.

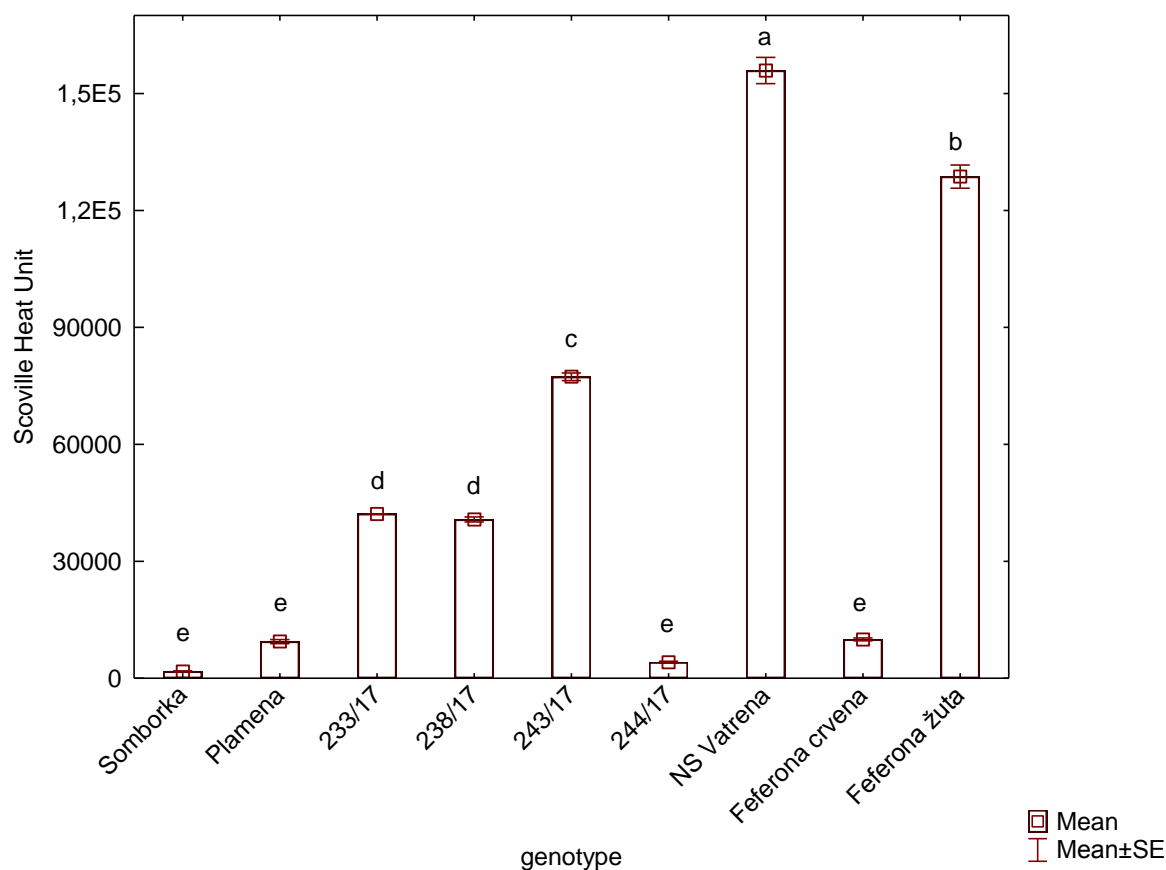


Figure 3. Scoville Heat Units in hot pepper genotypes.

Different letters show significant differences between genotypes ($P < 0.05$) according to the Bonferroni test.

CONCLUSIONS

The present study reports about morphological traits, capsaicin and dihydrocapsaicin contents and pungencies in SHUs for 9 genotypes (5 varieties and 4 breeding lines) of hot peppers in technological maturity. Despite the small number of tested genotypes, obtained data showed high diversity of measured traits, particularly in pungency level. The level of capsaicin varied from 47-5312 mg kg⁻¹ of dry matter, while dihydrocapsaicin varied from 62-4981 mg kg⁻¹ of dry matter. According to the Scoville scale, peppers were classified from mildly pungent to very highly pungent (1754-155896 SHU). Screening of two main capsinoids in domestic Serbian varieties and breeding lines of hot pepper could be a good basis for further breeding program in Institute of Field and Vegetable Crops.

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