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COMPARATIVE ANALYSIS OF CAPSAICIN CONTENT IN PEPPERS (*CAPSICUM ANNUUM* L.) GROWN IN CONVENTIONAL AND ORGANIC AGRICULTURAL SYSTEMS

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Abstract

According to their chemical structure capsaicinoids are phenethylamine group of alkaloids and they are produced exclusively in the secondary metabolism of genus *Capsicum*, fam. Solanaceae. Pepper (*Capsicum annuum* L.) is economically one of the most important vegetable crop in the Republic of Macedonia. There is no methodical and long-term research in organic production of vegetables in the Republic of Macedonia until today. Still, largely speaking, there are current debates about the advantage of organic production as compared to conventional one in terms of morphology of products and especially their quality characteristics.

The aim of this paper is comparative analysis of capsaicin content in fruit extracts from (*Capsicum annuum* L.) produced in organic and conventional cultivation systems. Pepper genotypes Strumicka Kapija, Strumicka Vezena, Piran, Zupska Rana, Duga Bela and Kurtovska Kapija were subject of this study. Methanol ($\geq 99.9\%$) was used as capsaicin solvent for extraction of capsaicin from dried pepper fruit material. The determination of capsaicin content was performed by chromatographic analyses with High Pressure Liquid Chromatography (HPLC).

Organically grown pepper fruits were characterized with higher capsaicin content than the conventional one. The genotype Strumicka Vezena was characterized with the highest capsaicin content in the both cultivation systems.

Key words: *Capsicum annuum* L., pepper, capsaicin, organic cultivation system, conventional cultivation system

INTRODUCTION

Peppers are grown because of their fruits which are used in human nutrition all year round as fresh, roasted, marinated for different dishes and salads, pickled, preserved, paste, juices and powder paprika spice. Since fruits contain capsaicin, it is used in pharmaceutical industry (Jankuloski, 1997; Bosland et al., 2012).

Organic agricultural production is an integral part of the sustainable agricultural development and it is well established in practice in the past years in Republic of Macedonia. Although the organic production is practiced on only 1% of total arable land in the country, there is a growing trend of practicing organic production which gives an expectation that organic agriculture production might be a leader in future development for the rest of the

sustainable agriculture systems (National Plan for organic production, 2013-2030). As part of the sustainable agriculture, organic agriculture utilizes methods and practices which enhance soil fertility and in the same time minimize harmful impact on soil, water, air and health of farmers and consumers.

From the available literature, there is no methodical and long-term research in organic production of vegetables in Republic of Macedonia until today. Still, largely speaking, there are current debates about the advantage of organic production as compared to conventional one in terms of morphology of products and especially their quality characteristics.

This is a report about the first comparative

research in Republic of Macedonia on peppers (*Capsicum annuum* L.) grown in organic and conventional production systems.

The aim of this research is a comparative analysis of capsaicin content in extracts from fruits of six different pepper genotypes (*Capsicum annuum* L.) grown in organic and conventional system.

The capsaicin (8-Methyl-N-vanillyl-trans-6-nonenamide), a major metabolite in *Capsicum* species, is the most common representative of broad family of capsaicinoids. It is a strong and stable crystal alkaloid which does not change the characteristics on temperature, thus it keeps the original strength in long-terms when heated or frozen. It is produced mainly in the placenta of *Capsicum* fruits. Different content of capsaicinoids in pepper fruits gives diversity of their flavour, from sweet to very hot (De Witt, 1999; Tod and Catt, 2002; De, 2004; Maksimova et al, 2014). The capsaicin owns high antioxidative

activity which gives high recommendation for pepper consumption in everyday nutrition (Maksimova et al. 2013; Maksimova et al. 2014). The clinical trials have shown that the biological potential of capsaicin is due to its structure of alkaloid with different inhibitory effects: analgesic and redactor of pain (Bernstein, 1991; Tominaga, 1998; Cheng et al., 1999; Holt, 1999; Bunk, 2000; Ying-Yue et al., 2001; Košťálová, 2002); antimicrobial (Kurita, 2002); antibacterial (Kalia et al., 2012; Sinha et al., 2011; Ubulom et al., 2012); anticancer (Mori et al., 2006); cancerogenic (Dasgupta et al., 1998; Archer & Jones, 2002); anesthetic (Bernstein, 1991; De Witt, 1999; Cheng et al., 1999), cytostatic (Surh, 2002; Zhang et al., 2003). Even more, capsaicin can be used as biopesticide against the green peach aphid (*Myzus persicae* Sulz.) in organic production of different crops (Maksimova et al., 2013).

MATERIAL AND METHODS

Experiment site and design

The experiment was conducted on open field at Kamnik Bio Organic in Skopje during three successive years. The fields of Kamnik Bio Organic are certified for organic production since 2011.

Six different pepper genotypes were used as plant material in this study: Strumicka Kapija, Strumicka Vezena, Piran, Zupska Rana, Duga Bela and Kurtovska Kapija. All genotypes were sweet peppers, except Strumicka Vezena which is hot pepper.

The experiment was set up in completely randomized design in three replicates for both organic and conventional system. The experimental conventional and organic plots were in the near vacancy. Each genotype was represented with 45 plants in total for both production systems. During the vegetation period, conventional and organic production practices were accordingly applied. The described experimental design was applied during the three experimental years.

Plant material

Fruits from six genotypes of pepper (*Capsicum annuum* L.) grown under organic and conventional practices were used for determination of capsaicin content. For each extraction sample of five pepper fruits was used from each genotype, organically and conventionally grown respectively. Fruits from genotypes Strumicka Kapija, Kurtovska Kapija and Strumicka Vezena were collected

in botanical maturity (deep red color of fruits), while fruits from genotypes Piran, Zupska Rana and Duga Bela were collected in horticultural maturity (greenish to yellow color of the fruits). The phenological phenophases of fruit collection was chosen according to pepper genotype characteristics and their utilization for human consumption.

Extraction method

The fruit pericarp with placenta was dried on 40°C until constant weight in laboratory oven with proper ventilation system.

A plant tissue extractor with semipermeable bags for extraction was used in the extraction procedure. Pure methanol with High Pressure Liquid Chromatography (HPLC) grade ($\geq 99.9\%$)

was used as solvent; 2 g of dry pepper tissue was extracted with 5 ml pure methanol with HPLC grade. The extraction has been performed at room temperature, for 24 hours minutes in dark conditions. The extract was filtrated through 0.45 μ m filter before injection in High Pressure Liquid Chromatography (HPLC) instrument.

Instruments for capsaicin content determination

Determination of capsaicin content was performed on Varian Pro Star HPLC system (pump model 230, autosampler model 410, PDA detector model 330 and column thermostat model 500). The separation of capsaicin from the other extracted components was performed

with C18 column: Perkin Elmer C-18 150x4.6, 5 μ m. The mobile phase was methanol : water = 60:40 (v/v) with isocratic flow of 1.3 ml/min. Determination was performed on wavelength of 222 nm, injected volume 10 μ l for total analysis time of 20 min.

Preparation of standard dilutions of capsaicin

Standard stock solution of capsaicin with concentration of 650 mg/l was prepared in methanol with HPLC grade ($\geq 99.9\%$). Standards with concentrations 1.3 mg/l; 3.25 mg/l; 6.5 mg/l; 13.0 mg/l; 16.25 mg/l; 50 mg/l; 100 mg/l; 200 mg/l and 300 mg/l capsaicin in methanol were prepared from the standard

stock solution. For determination of the limit of detection (LOD) and limit of quantification (LOQ), we used calibration in low concentration area of calibration curve in range 1.3-13 mg/l. Maximum time for usage of the standards was one day.

Validation properties of the method

The calibration curve characteristics of applied methodology were $y=21567x+130756$ with correlation characteristics $p < 0.998$.

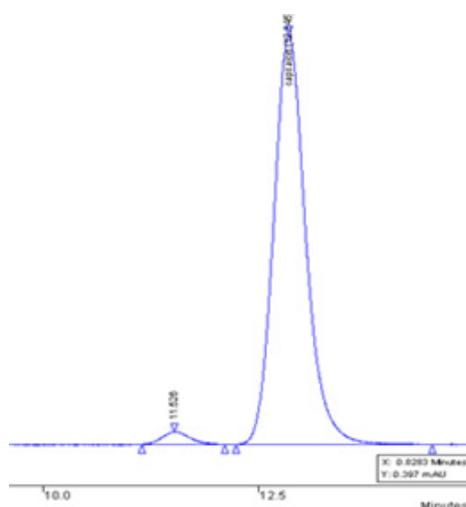


Figure 1. Chromatogram of standard capsaicin solution with concentration of $\gamma = 10$ mg/ml.

Detection of relative standard deviation (RSD) of the method is calculated from the calibration curve with extraction of 6 parallel repetition of pepper fruit samples from the same genotype and it is RSD 3%, while the LOD and LOQ are 0.5 mg/l and 1.5 mg/l, respectively (Fig. 1). Analytical yield of the method is calculated

from the calibration curve, where the analytical yield on both levels with two parallel repetition with standard addition is determined. The analytical yield in both cases < 95%.

The results of the capsaicin content in six different pungency pepper genotypes are expressed in mg capsaicin in dry matter.

Statistical analysis of data

The statistics of capsaicin content was done by the software programme IBM SPSS Statistics Software 19.0 (IBM SPSS Statistics 19 Brief Guide, 2010). The significance of differences between tested cultivation practices is established by the Duncan's Multiple Range Test. The evaluation of the effect of pepper genotypes and the effect of cultivation practices on capsaicin content

in the three years experiment was made by the statistical analysis of univariate for each pepper genotypes and both growing practices. Additionally, pairwise comparison was applied for estimation of significance between capsaicin content of the two applied cultivation practices.

RESULTS AND DISCUSSION

Many researchers reported High Pressure Liquid Chromatography (HPLC) as an excellent method for determination of capsaicin content in pepper fruits (Perucka & Oleszek, 2000; Othman et al., 2011; Reyes-Escogido et al., 2011).

The capsaicin content in the organic system varied from 0.31 mg/g (Kurtovska Kapija) to significantly the highest 9.57 mg/g (Strumicka Vezena) (Tab. 1).

On the other hand, the capsaicin content in the conventional production system ranged from 0.41 mg/g (Strumicka Kapija) to 7.22 mg/g (Strumicka Vezena) (Tab. 2).

As expected the hot genotype Strumicka

Vezena has shown the highest capsaicin content in both cultivation systems. The capsaicin content was influenced by the maturity of the fruits, since they were not harvest in the same maturity stage. Several studies have concluded that levels of capsaicinoids increase with maturation, remain constant or decrease slightly up to 60% after the maximum is reached. Yet, at all stages of growth, the total or individual capsaicinoids based on dry weight is far higher in the placenta than in the pericarp. This statement allows the conclusion that the main site of capsaicinoids synthesis is the placental tissue of the fruits (De, 2004).

Table 1. Capsaicin content in different pepper genotypes grown in organic production system.

Genotype	Capsaicin (mg/g dry matter)
Strumicka Kapija	3.96bc
Strumicka Vezena	9.57a
Piran	0.75c
Zupska Rana	1.24c
Duga Bela	0.79c
Kurtovska Kapija	0.31c

Means within the column having different letters are significantly different according to Duncan's test at $p < 0.05$.

Table 2. Capsaicin content in different pepper genotypes grown in conventional production system.

Genotype	Capsaicin (mg/g dry matter)
Strumicka Kapija	0.41c
Strumicka Vezena	7.22b
Piran	0.79c
Zupska Rana	2.33c
Duga Bela	0.62c
Kurtovska Kapija	0.47c

Means within the column having different letters are significantly different according to Duncan's test at $p < 0.05$.

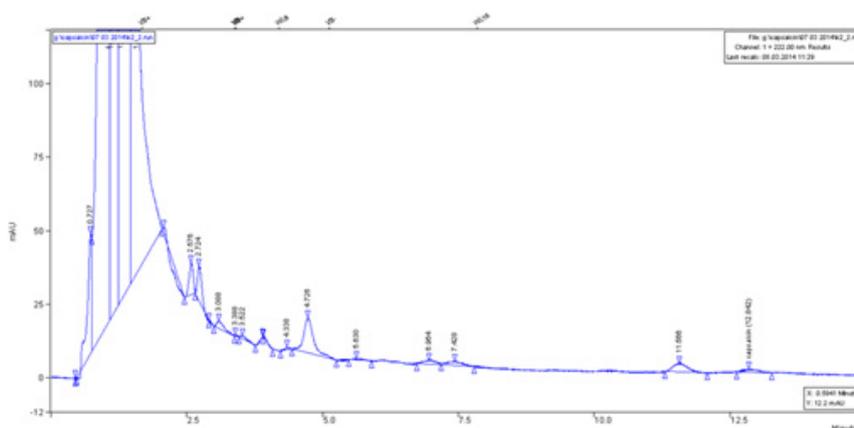


Figure 2. Chromatogram of analyzed sample of capsaicin content in Strumicka Kapija fruits.

The F test of the pepper genotypes effect on capsaicin content showed significant difference among tested genotypes during whole experiment, regardless cultivation practices applied (Tab. 3). When the same test was applied for the effect of cultivation practices

(organic and conventional) on capsaicin content in pepper fruits, capsaicin content was higher in fruits obtained by organic then by conventional cultivation practices in all pepper genotypes but without significant difference (Tab. 4).

Table 3. Univariate analysis of the effect of six pepper genotypes on capsaicin content in their fruits.

Dependent Variable: Capsaicin						
	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Pepper genotypes	551.095	5	110.219	13.349	.000	.527
Cultivation practice	11.464	1	11.464	1.388	.243	.023

The F tests the effect of **Pepper genotypes** and **Cultivation practice**. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Table 4. Pairwise comparison between capsaicin content in pepper fruits and cultivation system applied.

Dependent Variable: Capsaicin				
(A) Cultivation system	(B) Cultivation system	(A-B) Mean difference	Std. error	Significance
Organic	Conventional	.798	.677	.243

Estimated marginal means of capsaicin of tested genotypes in organic and conventional cultivation systems showed higher capsaicin

content in most of pepper genotypes under study (Figure 3).

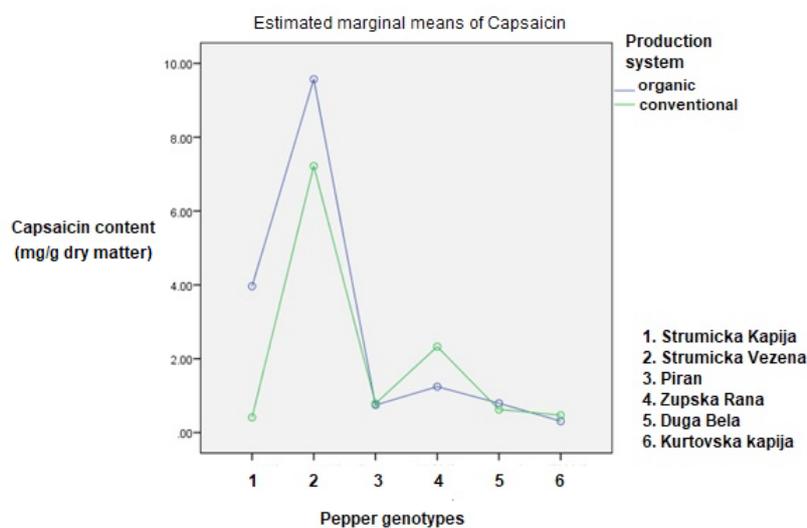


Figure 3. Estimated marginal means of capsaicin in six tested genotypes cultivated in organic and conventional production system.

Pepper fruits are rich with high number of bioactive compounds such as vitamin C and vitamin E, carotenoids and other polyphenolic compounds, but still the most of health beneficial and pharmaceutical properties and potential of peppers is due to capsaicin (Tominaga, 1998; Ying-Yue et al., 2001; Kurita 2002). There is a lack of research related to the content of nutritional and biologically active compounds as proteins, vitamins, ascorbic acid, minerals, fats and oils, phenolic compounds, aromatic substances in the fruits of varieties of *Capsicum* spp. in the Republic of Macedonia.

Maksimova et al. (2016) reported high capsaicin content in 13 hot pepper genotypes under study. Furthermore, they have not detected capsaicin in sweet pepper genotypes (Kurtovska Kapija and Zlaten Medal) when extraction was performed by maceration with 96% ethanol, which is opposite to our findings.

Simonovska et al., 2014 reported detailed composition of red hot pepper fruits including capsaicinoids content in pericarp (5.38 mg/g), seeds (2.36 mg/g) and placenta (10.48 mg/g) of pepper genotype Horgoshka grown in Macedonian conditions which is in agreement with capsaicin content found in the pericarp and placenta of Strumicka Vezena. However, capsaicin content varies depending on pepper genotype, cultivation conditions and fruit phenological stage (Maksimova et al., 2016; De, 2004; Govindarajan & Sathyanarayana, 1991). Kraikruan et al. (2008) informed that capsaicin and dihydrocapsaicin contents were the highest in the first harvest in all cultivars and then they decreased in the subsequent harvests. The highest capsaicin content in fruits was found in cultivars grown at a high temperature and in nutrient-rich soils (Sung et al., 2005; Rahman & Inden, 2012).

CONCLUSION REMARKS

The capsaicin content found in six pepper genotypes grown in organic and conventional cultivation systems varies due to pepper genotypes diversity and applied cultivation practices. The highest capsaicin content was found in Strumicka Vezena

grown in both cultivation systems. Overall, the capsaicin content found in organically cultivated genotypes was higher compared to conventionally grown which justifies the organic cultivation of pepper.

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КОМПАРАТИВНА АНАЛИЗА НА СОДРЖИНАТА НА КАПСАИЦИН ВО ПИПЕРКИ (*CAPSAICUM ANNUUM* L.) ОДГЛЕДУВАНИ ВО КОНВЕНЦИОНАЛЕН И ОРГАНСКИ СИСТЕМ

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Резиме

Капсаиноидите кои според својата хемиска структура припаѓаат кон фенилетиламинската група на алкалоиди се создаваат од секундарниот метаболизам исклучиво на растенијата од родот *Capsicum*, фамилија *Solanaceae*. Пиперката (*Capsicum annuum* L.) по своето стопанско значење е една од водечките градинарски култури во Република Македонија. Досега во Република Македонија нема методолошко и долготрајно истажување за органско производство на зеленчук. Сè уште се водат дебати во поширока смисла на зборот за предностите на органското производство во споредба со конвенционалното во однос на морфологијата на продуктите и посебно нивниот квалитет.

Целта на овој труд беше да се направи компаративна анализа на застапеноста на капсаицин во екстракти од плодови одгледувани во органски и конвенционални системи. Генотиповите на пиперки кои се опфатени во истражувањето се: *струмичка капија*, *струмичка везена*, *пиран*, *жупска рана*, *дуга бела* и *куртовска капија*. Како екстрактант на капсаициноот беше користен метанол ($\geq 99.9\%$) за екстракција на капсаициноот од сушени плодови на пиперка. Детерминацијата на содржината на капсаицин беше направена со хроматографска анализа со HPLC (високопритисочна течна хроматографија).

Плодовите од пиперка одгледувани во органско производство се одликуваа со висока содржина од конвенционалните. Генотипот *струмичка везена* се одликува со највисока содржина на капсаицин и во двата системи на производство.

Клучни зборови: *Capsicum annuum* L., *pepper*, *капсаицин*, *органски систем на одгледување*, *конвенционален систем на одгледување*.