DETERMINATION OF THE CONTENT OF CAPSAICIN AND DIHYDROCAPSAICIN IN TWELVE VARIETIES OF CHILLI PEPPERS USING LIQUID CHROMATOGRAPHY WITH UV/VIS DETECTION

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Abstract: The aim of the experiment was to determine the content of capsaicin and dihydrocapsaicin in twelve varieties of chilli peppers (Brown Bhutlah II, Bhut Jolokia Yellow I, Pieto de Moca II, Trinidad 7 POT II, 7 POT White II, Naga Jolokia I, Habanero Orange, Naga Viper II, Bhut Jolokia II, Jalapeno, White Naga Bhut Jolokia I, Carolina Reaper II) using high performance liquid chromatography UltiMate 3000 with UV/VIS detector. Each variety of chilli peppers was prepared without seeds and partitions (septa) and with seeds and partitions (septa) in order to compare yields of capsaicin and dihydrocapsaicin depending on the sample preparation. Based on the experiment, it was shown that chilli peppers with seeds and partitions (septa) had a higher content of capsaicin and dihydrocapsaicin compared to chilli peppers without seeds and partitions (septa). The highest capsaicin and dihydrocapsaicin content was of chilli peppers Carolina Reaper II. From the contents of capsaicin and dihydrocapsaicin was calculated so-called pungency in Scoville units (SHU) in order to compare our results with previous studies.

Key Words: capsaicin, dihydrocapsaicin, high performance liquid chromatography, UV/VIS detection

INTRODUCTION

Chilli peppers are the pungent fruits from plants of genus *Capsicum* in the nightshade family, *Solanaceae*, including 27 wild species and 5 main domestic species: *Capsicum frutescens*, *Capsicum pubescens*, *Capsicum chinense*, *Capsicum annuum*, *Capsicum baccatum* (Gurnani et al. 2016, Nickels 2015). The fruits are commonly used to give a pungent or hot sensation too many different dishes and food products all around the world (Duelund and Mouritsen 2017). The chilli peppers can be used also to produce chilli oil or chilli resin also known as oleoresin (Fernandez-Ronco et al. 2011).

The compounds responsible for the pungency of the chilli peppers belong to group of secondary metabolites known as capsaicinoids. Two major capsaicinoids found in chillies are capsaicin and dihydrocapsaicin. Capsaicin and dihydrocapsaicin represent about 77–98% of total capsaicinoids content in peppers. Other minor capsaicinoids found in chillies are nordihydrocapsaicin and homocapsaicin (Duelund and Mouritsen 2017, Sarpras et al. 2016). Capsaicin was discovered by P. A. Buchholtz in 1816. In 1846 L. T. Tresh isolated the capsaicin in a crystalline state and it was him who named the pungent substance capsaicin (DeWitt and Bosland 2009). Dihydrocapsaicin was described in 1957 (De 2004). Capsaicin is a very stable alkaloid, which is stable under exposure to heat and cold, has no flavour, colour, aroma and is insoluble in water, but easily soluble in fat or some organic solvents (DeWitt and Bosland 2009). Capsaicin is known for his pharmacological, neurological and dietetic effectiveness. It has also significant antibiotic activity and the ability to reduce the cholesterol level in blood (Gurnani et al. 2016). Capsaicin is also capable to kill some types of cancer cells (Anandakumar et al. 2013, Dawan et al. 2017, Prasad et al. 2008) and provide

relief in arthritis and respiratory ailments (Prasad et al. 2008), it is also antioxidant and has anticancer, antiarthritic and analgesic properties (Dawan et al. 2017, Prasad et al. 2008).

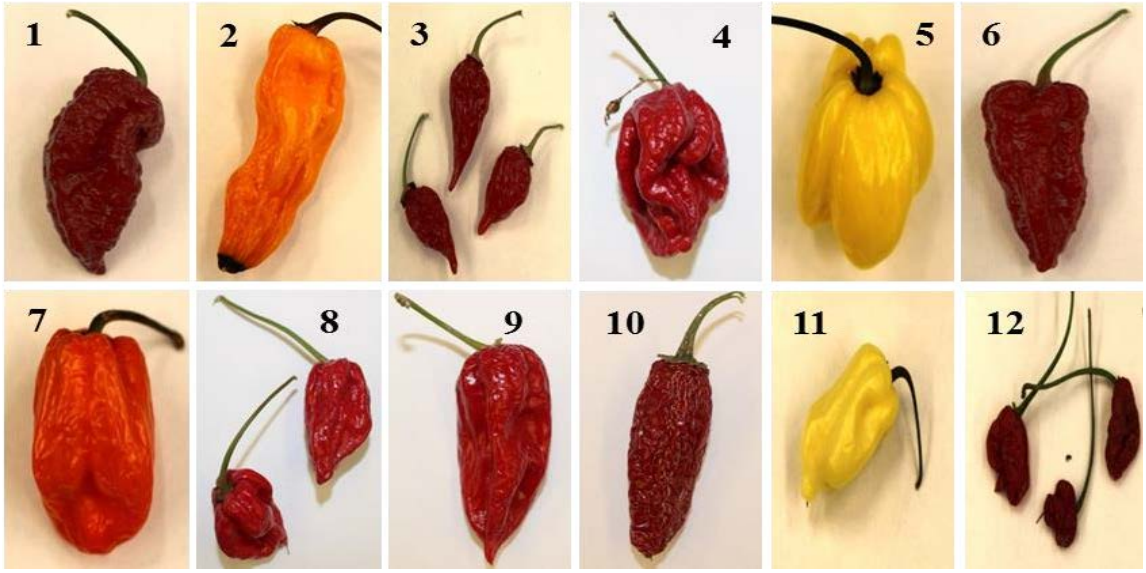
The capsaicin biosynthesis involves two pathways, i.e. the phenylpropanoid pathway, which provides the precursor phenylalanine for the formation of vanillylamine, and the valine pathway which provides the precursor valine for 8-methyl-6-nonanoic acid. Capsaicin is biosynthesized by capsaicin synthetase (CS) through condensation of products of the phenylpropanoid and valine pathways (Prasad et al. 2008). Capsaicinoids accumulation is found specifically in the epidermal layer called dissepiment of the placental tissue (Kybal 1988, Prasad et al. 2008, Sarpras et al. 2016).

The amount of capsaicinoids in chilli peppers depends on variety, age, degrees of maturity, season and agronomic conditions. Generally, in the bigger fruits there is less content of capsaicinoids than in the smaller fruits. Drying affects the content of capsaicinoids (Maradova 2015). Between the colour and amount of chillies there is no relationship (Nickels 2015). The pungency of chilli peppers is expressed by Scoville Heat Units (SHU). Scoville scale was invented by scientist Wilbur Lincoln Scoville in 1912 when he used the organoleptic test. In this test, a certain amount of chilli is extracted with ethanol, which then is diluted repeatedly until the pungent sensation no longer can be detect on the tongue. The number of times the extract has to be diluted is then taken as the pungency in units of SHU (Bosland and Votava 1999, Nickels 2015). Currently the content of capsaicinoids in chilli peppers is measured by HPLC (high-performance liquid chromatography) or GC (gas chromatography) (Duelund and Mouritsen 2017).

MATERIAL AND METHODS

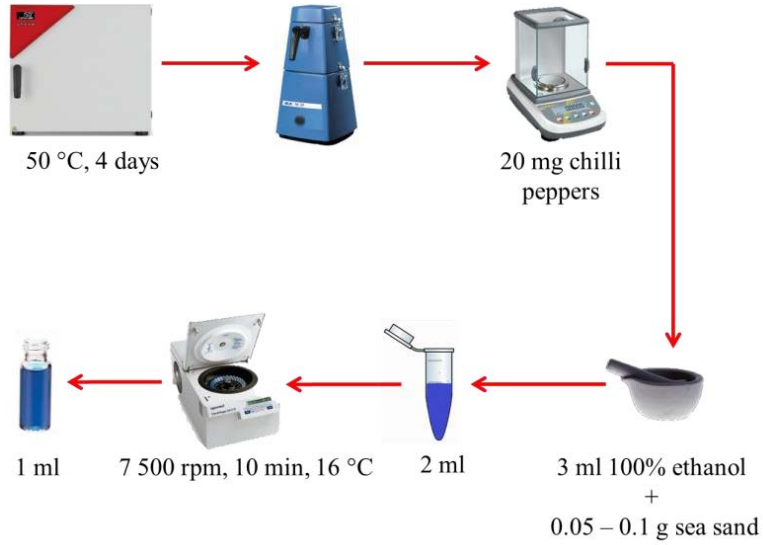
In this experiment twelve varieties of chilli peppers were used (Figure 1).

Figure 1 Varieties of chilli peppers: (1) Brown Bhutlah II, (2) Bhut Jolokia Yellow I, (3) Pieto de Moca II, (4) Trinidad 7 POT II, (5) 7 POT White II, (6) Naga Jolokia I, (7) Habanero Orange, (8) Naga Viper II, (9) Bhut Jolokia II, (10) Jalapeno, (11) White Naga Bhut Jolokia I, (12) Carolina Reaper II.



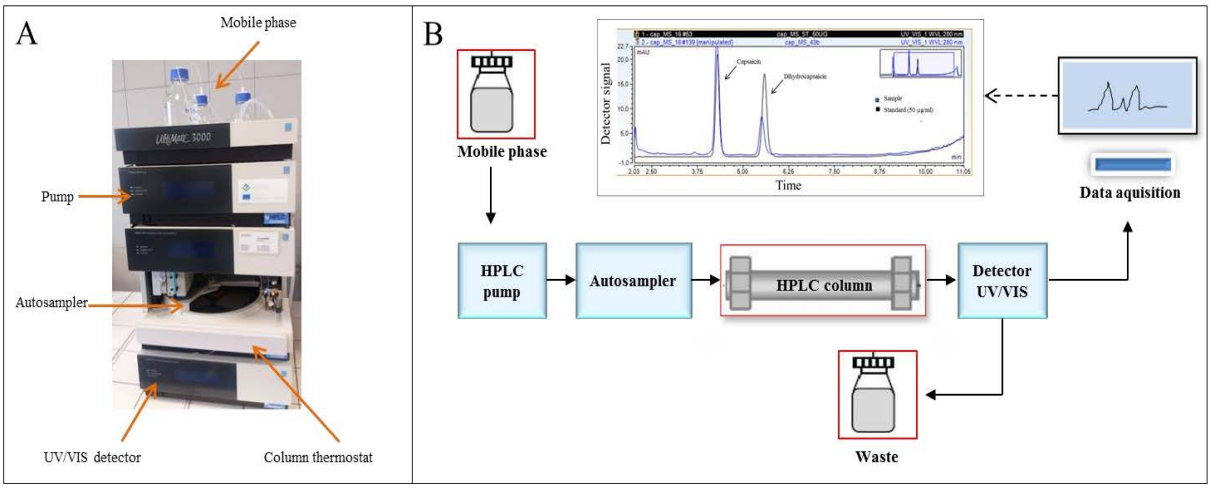
Sample preparation is described in Figure 2. At first, chilli peppers were divided into two parts. The first part of samples has got rid of seeds and partitions whereas in the second part of samples seeds and partitions were preserved. These samples were dried 4 days at 50 ºC (Drying and heating chamber BINDER ED 56, Verkon, Czech Republic). After drying the samples were milled (IKA M20 Universal mill) and from each sample was weighted 20 mg (Analytical Balance EP 240A, Precisa, Czech Republic). Then weighted samples were subsequently homogenized with 3 ml 100% ethanol and 0.05–0.1 g sea sand in friction bowl. Then homogenate was transferred to the microtube (Eppendorf, Germany) and centrifuged at 7 500 rpm at 16 ºC for 10 min. Finally, 1 ml of supernatant was collected from each sample to glass vials (Chromservis s. r. o., Czech Republic).

Figure 2 Scheme of preparing samples of chilli peppers for HPLC analysis.



The capsaicin and dihydrocapsaicin was determined by HPCL UltiMate 3000 (ThermoFisher Scientific, Waltham, USA) with UV/VIS detector (UltiMate 3000 Variable Wavelength Detector). The scheme of high performance liquid chromatograph is shown in Figure 3.

*Figure 3 (**A**) HPLC UltiMate 3000 (**B**) Scheme of high performance liquid chromatograph with UV/VIS detection.*



For the separation of capsaicin and dihydrocapsaicin, the column HYPERSIL GOLD DIM with dimensions 150 x 4.6 mm and a particle size of 5.0 µm was used. The column was equilibrated at 30 °C. Mobile phase A was 0.2% acetic acid and mobile phase B was 100% methanol. Flow rate of mobile phase was 1.0 ml/min. The compounds were eluted with a linear upward gradient: 0–6 min (70% B) 6–9 min (70–100% B) 9–12 min (70% B). The separated substances were detected at a wavelength of 280 nm. Calibration curves for contents of capsaicin and dihydrocapsaicin were linear from 1.00 to 500.00 µg/ml, with correlation coefficients of 0.9989 for both capsaicinoids of chilli peppers without seeds and partitions and 1.00 for both capsaicinoids of chilli peppers with seeds and partitions. All used chemicals were purchased from Sigma-Aldrich (St. Louis, USA). Capsaicinoids contents were re-calculated to Scoville heat units (SHU) by multiplying the pepper dry weight capsaicinoids concentration in parts per million (ppm) by the coefficient of the heat value for each compound. The coefficients are 16.0 for both capsaicin and dihydrocapsaicin.

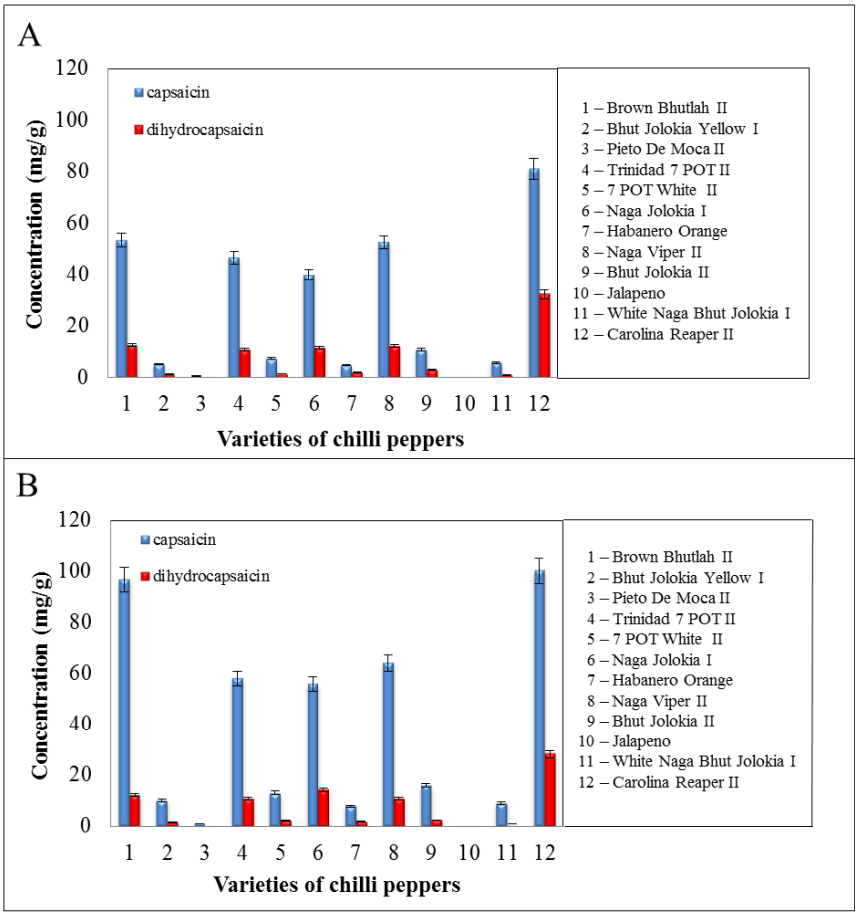
Statistical analyses

Content of capsaicin and dihydrocapsaicin in twelve varieties of chilli peppers were made using standard deviation from three determinations.

RESULTS AND DISCUSSION

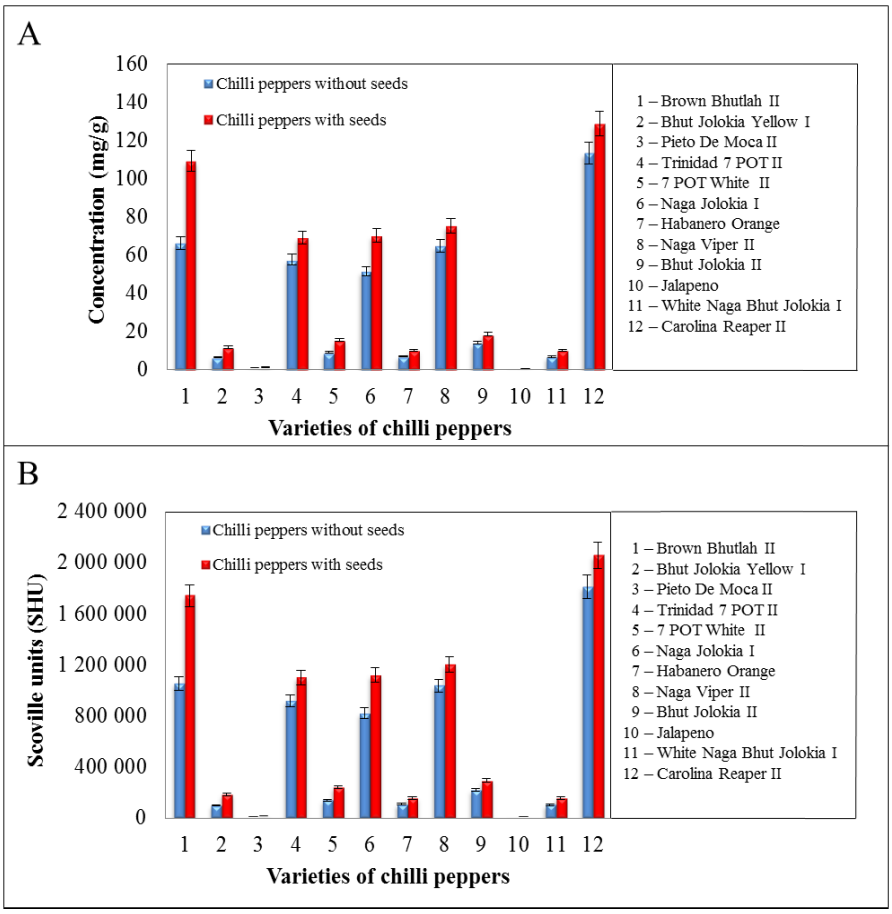
Determination of the presence and content of capsaicin and dihydrocapsaicin were done by high performance liquid chromatography with UV/VIS detection. The results have been recalculated per 1 g of chilli pepper.

*Figure 4 Determination of capsaicin and dihydrocapsaicin (**A**) for the extract of chilli peppers without seeds and partitions, (**B**) for the extract of chilli peppers with seeds and partitions.*



For chilli peppers without seeds and partitions (Figure 4A) we found the highest content of capsaicin (81 ± 4 mg/g) and dihydrocapsaicin (32 ± 2 mg/g) in Carolina Reaper II. The lowest content was in Jalapeno (0.03 ± 0.002 mg/g capsaicin and 0.03 ± 0.002 mg/g dihydrocapsaicin). The contents of capsaicin and dihydrocapsaicin in chilli peppers with seeds and partitions are shown in Figure 4B. The highest content of capsaicin (100 ± 5 mg/g) and dihydrocapsaicin (28 ± 1 mg/g) in chilli peppers with seeds and partitions also was in Carolina Reaper II. The content of capsaicin has increased in all chilli peppers with seeds and partitions whereas the content of dihydrocapsaicin was very various. The content of dihydrocapsaicin slightly increased in four varieties of chilli peppers with seeds and partitions (Bhut Jolokia Yellow I, 7 POT White II, Naga Jolokia I, Jalapeno), in rest of varieties we found less dihydrocapsaicin except Jalapeno. Jalapeno had same level of dihydrocapsaicin in chilli pepper with seeds and without seeds and partitions. Similar results of content of capsaicin and dihydrocapsaicin in Jalapeno were reported in study of (Pena-Alvarez et al. 2009) where, was used different sample preparation and method of determination.

*Figure 5 Determination of total capsaicinoids (**A**) and determination of Scoville units (**B**).*



In Figure 5A there is shown the concentrations of total capsaicinoids (capsaicin + dihydrocapsaicin) in chilli peppers with seeds and partitions and without seeds and partitions. From the figure 5A it is obvious the total content of capsaicinoids was higher in chilli peppers with seeds and partitions than in chilli peppers without seeds and partitions. The biggest difference was in Jalapeno where the content of total capsaicinoids increased by 739%. The least variance was in varieties of Carolina Reaper II (13%) and Naga Viper II (16%). For the remaining varieties, capsaicinoids increased in range from 20 to 84%. From the contents of capsaicinoids was calculated so-called pungency in Scoville units (SHU) (Figure 5B). Recently study (Duelund and Mouritsen 2017) found the pungency of Habanero 247 000 ± 24 000 SHU and Carolina Reaper 1 046 000 ± 34 000 SHU. We found a pungency of Habanero Orange with seeds and partitions 157 837 ± 7 892 SHU and Carolina Reaper II 2 056 026 ± 102 800 SHU.

CONCLUSION

Based on the results of measurement of capsaicin and dihydrocapsaicin we can conclude, that the total content of capsaicinoids depends on a sample preparation, whether extract is prepared from chilli pepper with or without seeds and partitions. The amount of capsaicinoids in chilli peppers also depends on variety, age, degrees of maturity, season and agronomic conditions. The highest concentration of capsaicin and dihydrocapsaicin was determined in Carolina Reaper II so it means it was also the most pungent chilli pepper.

Our future aim will be to test other sample preparation and monitor the change in the concentration of capsaicin and dihydrocapsaicin.

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