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Bioactive compounds and antioxidant activity of hot pepper fruits at different stages of growth and ripening

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Summary

The evolution of some bioactive compounds and antioxidant activity has been investigated during fruit growth and ripening of five pepper cultivars: 'Dracula', 'Pintea', 'Pepperone', 'Bulgarian carrot' (*C. annuum*) and 'Christmas bell' (*C. baccatum* var. *pendulum*). High-performance liquid chromatography was used to quantify the content of capsaicin in the fruit in order to determine the pungency level of analyzed peppers. Pepper fruits were collected at five stages of growth and ripening. Dry matter, soluble solids, ascorbic acid, total phenolics, including total flavonoids, capsaicin content and antioxidant activity were determined at each stage. There were major differences among the cultivars in the accumulation of the bioactive compounds in the fruit during their growth and ripening, although the quantitative accumulation pathway of various components had a similar trend during phenophases. Antioxidant activity and ascorbic acid content increased during growth and ripening of hot peppers, the highest levels being found in the last stage of ripening. The pattern of variation of total flavonoid content was cultivar dependent. In most cultivars, an important increase of the total phenolic and total flavonoid content was observed in the last stage of ripening. Capsaicin content recorded a maximum level in F3 or F4 depending on cultivar, and decreased afterwards until the complete ripening of the pepper fruits. 'Dracula' cultivar was classified as "non-pungent" (fruits are not spicy) while 'Pintea' was classified as "highly pungent", the other analyzed cultivars having an average level of pungency.

Keywords: hot pepper, antioxidant activity, phenolics, flavonoids, capsaicin

Introduction

Hot pepper was part of ancient human diet in the American continent since 7500 years BC. Its South American origin is supported for all species of *Capsicum* genus, which probably occurred in the region between southern Brazil and Bolivia.

Hot pepper has a sweet-pungent flavor, with pungency ranging by cultivar. Peppers are good sources of phytochemicals such as polyphenols, among them flavonoids, and carotenoids (ALVAREZ-PARRILLA et al., 2010) that are known to present high antioxidant activity (MATTERSKA and PERUCKA, 2005). Phenolic compounds from pepper contribute to nutritional quality and fruit taste (ORNELAS-PAZ et al., 2010).

The substance that gives peppers pungency is capsaicin that is found in very small quantities in sweet pepper and in quantities of tens and hundreds of times higher in hot peppers. As the content of capsaicin in peppers increases, so does its pungent taste, which triggers an increase in their antioxidant level (USMAN et al., 2014). Therewith capsaicin controls the appetite and raises the body tem-

perature (LUDY and MATTES, 2011). Pungency is measured with the dilution test and expressed as Scoville Organoleptic Scale designed by WILBUR SCOVILLE in 1912. Recognized as the most pungent pepper (World-record for hottest chili of 2012) is *Trinidad Moruga Scorpion*, 2.000.000 Scoville Units higher than any other species. Recognized as the less spicy peppers are *Pimento*, *Peperoncini* and *Banana pepper* cultivars, with only 100-500 Scoville Units. In addition to capsaicin, chili peppers' taste is given by their content of essential oils. Capsaicin and dihydrocapsaicin represent about 75-90% of capsaicinoids in peppers, compounds that also have other properties and biological effects, such as chemopreventive (CHANDA et al., 2004) or antioxidant properties (ZHUANG et al., 2012). The epidermal cell of the placenta seems to be the accumulation site of capsaicinoids in peppers (BARBERO et al., 2014). Capsaicinoids are found only in the *Capsicum* genus and their amounts varies in different pepper varieties depending on cultural practices, environmental conditions, storage conditions, etc. (USMAN et al., 2014). Literature mentions that dry matter and soluble solids content of pepper and different compounds in pepper, such as ascorbic acid and capsaicinoids vary during growing and ripening of the fruit (BARBERO et al., 2014; MARTINEZ et al., 2007).

This work was conducted to investigate the evolution of some bioactive compounds and antioxidant activity of five pepper varieties grown in Dolj County, Romania during fruit growth and ripening.

This work also aims to estimate the evolution and the level of capsaicin in fruit and to determine the pungency level of analyzed peppers.

Materials and methods

Plant material

Fruits of five pepper cultivars (*Capsicum annuum* and *Capsicum baccatum*): 'Dracula', 'Pintea', 'Pepperone', 'Bulgarian carrot' (*C. annuum*) and 'Christmas bell' (*C. baccatum* var. *pendulum*), were collected at five stages of development and ripening i.e. F1-14 days after flowering, F2-20 days after flowering, F3-30 days after flowering, F4-40 days after flowering, F5-50 days after flowering (physiological maturity) from Dabuleni, Dolj county, Romania and analyzed in terms of chemical characteristics and antioxidant activity. The selection of the fruits was based on the number of days after flowering.

Dabuleni is a sandy area in the South of Romania close to the Danube River. It has a strong continental climate with mild Mediterranean influence and an average temperature of 11 °C. The region gets severe drought from July to September and average rainfalls in May and June. The orchard management was consistent with cultural practice (10-12 watering sessions with 300-350 m³/ha, 100-100-50 NPK fertilization, breaking the tip of plant, pests and diseases control). The planting was carried out in sandy soil, with the experiment being set up as randomized block design in 3 replicates with 30 plants per cultivar. From each cultivar 10 fruits in 3 replicates were collected in order to perform chemical analysis.

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Analytical methods

Several analyses have been performed: dry matter, soluble solids, ascorbic acid, total phenolic and total flavonoid content, capsaicin content and antioxidant activity. The dry matter content was determined by removing water from the sample in an oven at 105 °C and the result was expressed in percentages. The soluble solids were measured with a digital refractometer (Hanna Instruments, Woonsocket, USA) from the juice pressed from the fruit, the results being expressed in percentages.

Ascorbic acid content

Ascorbic acid was extracted and analyzed by reversed phase HPLC. Fresh pepper homogenate (5 g) was mixed and diluted with 2% HCl. After 20 minutes the solution was centrifuged at 4200 rpm for 10 min. The supernatant was filtered through 0.2 mm pore size filter. The ascorbic acid in the sample test solution was separated by reversed phase chromatography on a 250 mm × 4.6 mm i.d., 5 µm particle Hypersil Gold aQ Analytical Column, of which was detected by absorbance and quantified with external calibration graph. The detector was set at $\lambda=254$ nm. This setting was chosen since ascorbic acid has its maximum optical absorbance close to 254 nm. The HPLC analysis was performed with a Surveyor Thermo Electron system (Thermo Electron Corporation, San Jose, CA, USA) comprising a vacuum degasser, Surveyor Plus LCPMPP pump, Surveyor Plus ASP autosampler and diode array detector with 5 cm flow cell. Integration, data storage and processing were performed by Chrom Quest 4.2 software. The determinations were made in isocratic conditions, at 10 °C, using a mobile phase made of 50 mM phosphate solution filtered through a polyamide membrane (0.2 µm) and degassed in a vacuum. The flow rate of the mobile phase was 0.7 mL/min for all the chromatographic separations. The volume injected was 5 µL for either prepared sample or standard solution.

The results were expressed in mg kg⁻¹ fresh weight (fw). All reagents were acquired from Sigma-Aldrich, Germany and ultrapure water was obtained from a Milli-Q water purification system (TGI Pure Water Systems, USA).

Total phenolic content

Total phenolic content was assessed by using the Folin-Ciocalteu phenol reagent method (SINGELTON and ROSSI, 1965). Folin-Ciocalteu reagent (2 N, Merck), gallic acid (99% purity, Sigma-Aldrich), anhydrous sodium carbonate (99% Sigma-Aldrich) were used. One g dried pepper homogenate was extracted with 15 mL ethanol in an ultrasonic bath for 60 min at ambient temperature. After extraction, the samples were centrifuged for 5 min at 4200 rpm and supernatants were filtered through polyamide membranes with pore diameter of 0.45 µm and stored at a temperature of -20 °C. Hundred µL of each pepper ethanolic extract were mixed with 5 mL of distilled water and 500 µL of Folin-Ciocalteu reagent. After 30 sec to 8 min, 1.5 mL of sodium carbonate (20% v/v) was added. The reaction mixture was diluted with distilled water to a final volume of 10 mL. The preparation of the standard solution of gallic acid followed the same procedure. The absorbance at 765 nm of each mixture was measured on a Varian Cary 50 UV spectrophotometer (Varian Co., USA) after incubation for 30 min at 40 °C and results were expressed in mg gallic acid (GAE) kg⁻¹ dry weight (dw).

Antioxidant activity

The antioxidant activity (AOX) was measured in the ethanolic extract using the DPPH (2,2-diphenyl-1-picrylhydrazil) assay. Ethanol (Merck, Germany), DPPH (2,2-diphenyl-1-picrylhydrazil) (Sigma-Aldrich, Germany) and Trolox (Merck, Germany) were employed. The extraction of samples was made according to the same proto-

col described for total phenolic content. The free radical scavenging ability of the extracts against DPPH free radical was evaluated as described by OLIVEIRA et al. (2008), with some modifications. Each ethanol pepper extract (50 µL) was mixed with 3 mL of a 0.004% (v/v) DPPH methanolic solution. The mixture was incubated for 30 min at room temperature in the dark and the absorbance was measured at 517 nm on Varian Cary 50 UV-VIS spectrophotometer. The DPPH free radical scavenging ability was calculated in reference to Trolox (6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid), which was used as standard reference to convert the inhibition capability of each extract solution to the mmol Trolox equivalent antioxidant activity/L. The radical was freshly prepared and protected from light. A blank control of methanol/water was used in each assay. All assays were conducted in triplicate and results were expressed in mmol Trolox kg⁻¹ dw.

Total flavonoid content

Total flavonoids content was assessed by the spectrophotometric method described by MOHAMMADZADEH et al. (2007) based on the color reaction of this class of compounds with the ions of Al (III). Pepper homogenate (1 g dry matter) was extracted with 15 mL ethanol in an ultrasonic bath for 60 min at ambient temperature. After extraction, the samples were centrifuged for 5 min at 4200 rpm and supernatants were filtered through polyamide membranes with a pore diameter of 0.45 µm and stored at -20 °C. 0.5 mL filtrate was placed in a polyethylene test tube, together with a 0.1 mL 10% aqueous solution of aluminum nitrate, 0.1 mL aqueous 1 M sodium acetate, 4.3 mL methanol, mixed well and left to react for 40 min at room temperature. After completion of the reaction, the absorbance of the mixture was measured at 415 nm on a Varian Cary 50 UV-VIS spectrophotometer (Varian Co., USA). The quantification of flavonoids was carried out on the basis of a calibration curve using quercetin as standard reference in the range of 0-100 mg/L. The results were expressed as mg of quercetin equivalents kg⁻¹ dw.

Capsaicin content

Identification and quantification of capsaicin were carried out by HPLC following the method described by ZHUANG et al. (2012), with some modifications.

For sample preparation, fresh matter (pulp transformed into a paste by a disintegrator) was dried in an oven at 55 °C to a constant weight and the dried matter was ground finely in a blender (Polytron). An amount of 1 g of the dried matter was extracted with 20 mL of ethanol 95%. After 30 min, the extract was kept in an ultrasonic bath for 60 min at 60 °C and filtered through a 0.2 mm pore size filter.

HPLC-DAD analysis was performed on a Finnigan Surveyor Plus system (Thermo Electron Corporation, San Jose, CA, USA) coupled with diode array detector (PDA5P with cell flow in 5 cm). The separation was performed using a Hypersil Gold aQ column (250 × 4.6 mm) with a particle size of 5 µm. Capsaicin was detected at 278 nm and quantified (mg kg⁻¹ dw) using standard compound.

The determinations were made in isocratic conditions at 25 °C, using a mobile phase made of 50 mM acetonitrile solution (water:acetonitrile 57:43) filtered through a polyamide membrane (0.2 µm) and degassed in a vacuum. The flow rate of the mobile phase was 1 mL min⁻¹ for all the chromatographic separations. The volume injected was 5 µL for either prepared sample or standard solution. For device control, data acquisition and processing, Chrom Quest 4.2 software was used.

Statistical analysis

Significance of differences between cultivars was determined by performing a one-way ANOVA test using Statgraphics Centurion XVI Software (StatPoint Technologies, Warenton, VA, USA).

Results and discussion

The data obtained concerning the dry matter, soluble solids and ascorbic acid content of the hot peppers are presented in Tab. 1.

The dry matter content (%) in hot peppers followed an upward trend during growth and ripening except the F2 (20 days after flowering) where a small absolute decrease was noticed. However, the dry matter content varied little in immature stages but showed high increase in ripening stages with a maximum in the physiological maturity, corresponding to the ripening process of fruits, which was manifested through the synthesis of metabolites with complex molecular structure. The same changes were reported by NIKLIS et al. (2002). There were also differences among cultivars, the highest content of dry matter being found in the 'Pepperone' cultivar while the 'Pintea' cultivar registered the lowest content. The differences among cultivars were maintained during all the stages of growth and ripening. Regarding the soluble solids content (%), it was found that it had the same upward trend during growth and ripening except the 'Pintea' cultivar which registered a small decrease in F2 and 'Bulgarian carrot' cultivar which registered the same decrease in F3. The highest rate of accumulation of soluble substances was found in the last stages with similar values to those found by DORJI et al. (2005). DORJI et al. also found that the highest changes in soluble solids happened in the last stages of ripening and in the firm red stage, respectively. There were differences among cultivars: the highest content of soluble solids being found in the 'Bulgarian carrot' (9.82%) followed by 'Pepperone' (9.65%), 'Dracula' (8.4%), 'Pintea' (8.1%) and 'Christmas bell' (7.40%).

In all cultivars the ascorbic acid content was positively correlated with the dry matter and the soluble solids content, which is in accordance with the data presented by NIKLIS et al. (2002).

Considering that the glucose is the precursor in the ascorbic acid synthesis (DAVEY et al., 2000; NIKLIS et al., 2002) it is understandable why there is a positive correlation between dry matter, soluble solids

and ascorbic acid content. The highest accumulation of ascorbic acid was recorded between the first phenophases (F1-F2). In this period in all cultivars high plant growth rates were observed.

The data obtained are comparable to data presented in the literature, the upward trend being also described by KUMAR and SUBBA TATA (2009) and MARTINEZ et al. (2005). There were significant differences among cultivars, the highest content of ascorbic acid being found in the 'Bulgarian carrot' cultivar (1606.47 mg kg⁻¹ fw) and the lowest content in the 'Pintea' (1161.35 mg kg⁻¹ fw) cultivar.

According to the classification of hot pepper cultivars depending on the content of ascorbic acid described by KHADI et al. (1987) and SIMONNE et al. (1997), the analyzed cultivars fall into the category of an average content of ascorbic acid (1010-2000 mg kg⁻¹ fw) even if analyzing in terms of species, chilli is considered a species with high ascorbic acid content.

The results obtained on the content of phenolics, total flavonoids, and antioxidant activity (AOX) in hot peppers are shown in Tab. 2.

Antioxidant activity is an important parameter to establish the health functionality of a food product and there are many methods used for its measurement (KAUR and KAPOOR, 2001). AOX increased during growth and ripening of the hot peppers, the highest levels being found in the last stage (F5). There were differences among cultivars, the highest AOX level being found in the 'Pintea' cultivar (20.04 mmol Trolox kg⁻¹ dw) and the lowest in the 'Dracula' cultivar. The differences between cultivars were also reported by ZHUANG et al. (2012), who mentioned some pepper extracts as effective electron donors.

Hot peppers contain several flavonoids in different forms including glycosides (BAE et al., 2012). The data presented in Tab. 2 show important variations in the content of phenolics and total flavonoids in hot peppers. The total phenolic content increases during growth and ripening with the highest level in the last stage of ripening, the results being in accordance with the data presented by ZHUANG et al. (2012) and DEEPA et al. (2007).

RIGHETTO et al. (2005) mentioned that the content of phenolics is

Tab. 1: Dry matter, soluble solids and ascorbic acid content in hot peppers during growth and ripening. Different letters within the same row indicated significant differences ($P < 0.05$) among cultivars

	Dracula	Pintea	Pepperone	Bulgarian carrot	Christmas bell
Dry matter (%)					
F1	10.11±1.41c	11.38±1.09b	11.80±1.41ab	12.39±1.36a	12.53±1.62a
F2	9.99±1.19c	10.65±1.17bc	10.50±1.29b	10.12±1.11bc	12.13±1.49a
F3	10.86±1.75c	11.47±1.37b	13.31±1.73a	10.57±1.26c	12.73±1.52ab
F4	15.96±2.01b	14.17±1.73bc	15.42±2.15b	18.00±2.52a	13.66±1.77c
F5	17.43±2.26b	16.16±2.02c	18.71±2.6a	18.09±2.48a	17.53±2.38ab
Soluble solids (%)					
F1	4.45±0.53b	5.45±0.65a	4.70±0.56ab	3.72±0.40c	3.86±0.41c
F2	4.67±0.61ab	4.37±0.48c	4.54±0.49b	5.10±0.58a	4.66±0.51ab
F3	5.64±0.69bc	5.82±0.62b	7.10±0.91a	4.57±0.54c	6.00±0.72ab
F4	7.77±0.93ab	4.80±0.52d	7.60±0.98b	8.32±1.05a	6.66±0.86c
F5	8.40±1.09b	8.10±1.03b	9.65±1.32ab	9.82±1.27a	7.40±0.96c
Ascorbic acid (mg kg ⁻¹ fw)					
F1	170.32±15.40a	152.80±12.80b	169.85±14.20ab	173.62±13.60a	105.43±90.80c
F2	1186.54±108.70a	1102.38±97.30d	1188.21±99.10a	1178.89±86.70b	1158.40±109.20c
F3	1104.62±100.40c	1123.19±104.10bc	1191.05±110.50b	1289.21±116.90a	1259.47±114.90a
F4	1211.20±109.60c	1149.52±98.30de	1176.23±105.80d	1297.58±113.70b	1393.62±121.40a
F5	1383.09±118.70c	1161.35±106.10d	1387.11±120.80bc	1603.57±147.50a	1446.47±137.30b

Tab. 2: Phenolics, total flavonoids, capsaicin content and antioxidant activity in hot peppers during growth and ripening. Different letters within the same row indicated significant differences ($P < 0.05$) among cultivars

	Dracula	Pintea	Pepperone	Bulgarian carrot	Christmas bell
Antioxidant activity (mmol Trolox kg ⁻¹ dw)					
F1	5.26±0.49c	6.94±0.58a	5.95±0.53bc	7.08±0.68a	6.24±0.60b
F2	7.47±0.61b	8.64±0.611a	7.36±0.71b	8.89±0.86a	7.14±0.68c
F3	9.01±0.88c	18.03±1.74a	11.58±1.08b	11.85±1.94b	6.91±0.67d
F4	9.49±0.90c	20.04±1.99a	12.55±1.92bc	13.87±1.26b	8.44±0.82d
F5	10.51±0.97cd	19.56±1.73a	17.50±1.67b	17.23±1.66b	11.55±1.09c
Total phenolics (mg GAE kg ⁻¹ dw)					
F1	3074.32±284.12b	3210.27±301.23a	3087.00±287.65ab	3299.95±301.11a	2768.23±265.34c
F2	3358.95±297.25b	3253.23±298.73bc	3462.78±300.87b	3779.28±369.30a	2878.89±279.61c
F3	3450.00±300.13cd	4965.69±405.87a	3525.13±312.65c	3758.11±358.49b	3267.67±311.42d
F4	3645.58±315.09d	7026.63±691.45a	4435.05±403.12c	6476.10±609.28b	3248.35±311.73d
F5	5146.45±463.67c	8331.27±786.70a	6433.51±503.84b	2964.25±275.33c	3744.84±350.22d
Total flavonoids (mg quercetin equivalents kg ⁻¹ dw)					
F1	1896.90±176.55c	1853.85±171.33c	2218.33±199.76bc	4532.89±432.05a	2415.15±230.17b
F2	1422.61±139.68d	1996.33±183.74c	2286.09±202.11b	4569.75±408.17a	2001.43±198.67b
F3	1868.20±183.15b	1819.65±167.95bc	1849.44±169.81b	3397.92±311.26a	1774.00±163.50c
F4	1632.97±156.70d	2726.10±222.56a	2340.39±201.54b	2382.73±202.88b	1844.23±187.34c
F5	5461.93±497.78b	5247.27±497.88bc	7666.09±700.80a	1435.57±122.86d	2848.81±256.40c
Capsaicin (mg kg ⁻¹ dw)					
F1	4.98±0.45c	37.57±3.28b	45.16±5.03a	0	0
F2	5.19±0.49c	705.90±56.91a	429.55±40.89b	11.10±0.98d	38.07±3.77e
F3	24.85±2.89e	2229.81±220.37a	777.49±75.69b	438.46±39.69c	126.78±11.59d
F4	16.89±1.56e	1814.86±179.65a	405.90±38.45c	990.37±93.15b	187.90±17.73d
F5	18.64±1.72e	1797.52±17.05a	501.07±48.71c	656.13±62.99b	30.46±2.86d

affected by the type and cultivar of pepper, maturity and cultural conditions which are in accordance with the data presented in Tab. 2, the highest content in phenolics (F5) being found in the 'Pintea' cultivar (8331.27 mg GAE kg⁻¹ dw) and the lowest in the 'Bulgarian carrot' cultivar (2964.25 mg GAE kg⁻¹ dw). The total flavonoids content presented different variation patterns from cultivar to cultivar but 30 days after flowering (F3) a decrease is recorded as compared with F1 in all cultivars. Except for 'Bulgarian carrot' cultivar, total flavonoids content grew strongly in the last stage of ripening. HOWARD et al. (2000) and MEDINA-JUAREZ et al. (2012) found the same variation associated to pepper maturity, cultivar and growing conditions.

In the last stage of ripening (F5) the total flavonoids content was significantly higher in the 'Pepperone' cultivar (7666.095 mg quercetin kg⁻¹ dw) followed by 'Pintea' and 'Dracula'.

The data regarding the capsaicin content of the hot pepper are presented in Tab. 2. In the early days after flowering (14 days) the capsaicin was found in peppers in very small amounts (undetectable in the 'Bulgarian carrot' and 'Christmas bell' cultivars).

During growth and ripening, the fruit content of capsaicin increased to a maximum level that was recorded depending on the cultivar precocity in F3 (30 days after flowering) in: 'Dracula', 'Pintea' and 'Pepperone' (early cultivars) either F4 (40 days after flowering) in 'Bulgarian carrot' and 'Christmas bell'. After reaching this peak, the capsaicin content decreased until the full ripening of the pepper fruits. BARBERO et al. (2014) mentioned that the maximum relative content of capsaicin is reached on day 20 of fruit maturation, earlier

than other capsaicinoids, and between day 40 and 50 of maturation the relative content of capsaicin represented only 52% of the total capsaicinoids. The same trend of capsaicin accumulation in fruits was reported by ESTRADA et al. (2000) and KIRSCHBAUM-TITZE et al. (2002).

There are major differences between cultivars regarding the content of capsaicin, the highest level being found in the 'Pintea' cultivar (2229.81 mg kg⁻¹ dw) with an equivalent pungency of 35676.96 SHU followed by the 'Bulgarian carrot' and 'Pepperone'. The lowest level of capsaicin content was found in the 'Dracula' cultivar (24.85 mg kg⁻¹ dw) with an equivalent pungency of 397.68 SHU. Similar differences between cultivars were reported by USMAN et al. (2014), VERA-GUZMÁN et al. (2011) and GNAYFEED et al. (2001).

To express the pungency level of the studied cultivars, capsaicin content (grams of capsaicin per grams pepper dry weight) was converted to Scoville Heat Units by multiplying it by the coefficient of the heat value (1.6×10^7) (TILAHUN et al., 2013). The data obtained are shown in Tab. 3.

Scoville score analysis showed that the 'Dracula' cultivar is classified as 'non-pungent' (fruits are not spicy) while 'Pintea' is classified as 'highly pungent', the other analyzed cultivars being classified as 'moderately pungent'.

Although the 'Pintea' cultivar registered the highest capsaicin content and the highest level of pungency, it is very far from the first places on the Scoville scale (Scoville units between 200,000 and 300,000 – *Capsicum chinense*, while very spicy peppers from Thailand only reach 100,000).

However we can say that the fruits of 'Pintea' cultivar are swifter than the Mexican Jalapeno or the Italian Peperoncino cultivars that barely reach a score of 500-5,000 Scoville Units (MATHUR et al., 2000).

Tab. 3: The pungency of the hot peppers

Cultivar	Maximum content of capsaicin mg kg ⁻¹ dw	Scoville Units SHU	Pungency
Dracula	24.85±2.89	397.68	Non-pungent (0-700 SHU)
Pintea	2229.81±220.37	35676.96	Highly pungent (25000-70000 SHU)
Pepperone	777.49±75.69	12439.92	Moderately pungent (3000-25000 SHU)
Bulgarian carrot	990.37±93.15	15846	Moderately pungent (3000-25000 SHU)
Christmas bell	187.90±17.73	3006.48	Moderately pungent (3000-25000 SHU)

Conclusions

There are major differences among cultivars in the accumulation of the bioactive compounds in the fruit during their growth and ripening, although the quantitative accumulation pathway of various components had a similar trend during phenophases.

The dry matter content varied little in immature stages but a large increase was observed at ripening stages with a maximum at the physiological maturity.

The soluble solids content had the same upward trend during growth and ripening and the ascorbic acid content was positively correlated with the dry matter and the soluble solids content.

Antioxidant activity increased during growth and ripening of hot peppers, the highest levels being found in the last stage of ripening.

Although the pattern of variation of total flavonoid content was affected by the cultivar, a lower value was recorded 30 days after flowering (F3) as compared with F1 in all cultivars. Also, in most cultivars, an important increase of the total phenolic and total flavonoid content was observed in the last stage of ripening.

Cultivar's greatest influence on the accumulation of bioactive compounds was observed regarding the content of capsaicin that was found in peppers in very small amounts (undetectable in the 'Bulgarian carrot' and 'Christmas bell' cultivars) in F1. During growth and ripening, fruit content of capsaicin increased to a maximum level (F3 or F4), then declined until the full ripening of the pepper fruits. Scoville score analysis showed that the 'Dracula' cultivar is classified as "non-pungent" (fruits are not spicy) while 'Pintea' is classified as "highly pungent", the other analyzed cultivars having an average level of pungency.

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References

ALVAREZ-PARRILLA, E., DE LA ROSA, L.A., AMAROWICZ, R., SHAHIDI, F., 2010: Antioxidant activity of fresh and processed Jalapeno and Serrano peppers. *J. Agric. Food Chem.* 59(1), 163-173. DOI: 10.1021/jf103434u

- BAE, H., JAYAPRAKASHA, G.K., JIFON, J., PATIL, B.S., 2012: Variation of anti-oxidant activity and the levels of bioactive compounds in lipophilic and hydrophilic extracts from hot pepper (*Capsicum* spp.) cultivars. *Food Chem.* 134(4), 1912-1918. DOI: 10.1016/j.foodchem.2012.03.108
- BARBERO, G.F., RUIZ, A.G., LIAZID, A., PALMA, M., VERA, J.C., BARROSO, C.G., 2014: Evolution of total and individual capsaicinoids in peppers during ripening of the Cayenne pepper plant (*Capsicum annuum* L.). *Food Chem.* 153, 200-206. DOI: 10.1016/j.foodchem.2013.12.068
- CHANDA, S., EREXSON, G., RIACH, C., INNES, D., STEVENSON, F., MURLI, H., BLEY, K., 2004: Genotoxicity studies with pure trans-capsaicin. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 557(1), 85-97. DOI: 10.1016/j.mrgentox.2003.10.001
- DAVEY, M.W., MONTAGU, M.V., INZÉ, D., SANMARTIN, M., KANELIS, A., SMIRNOFF, N., FLETCHER, J., 2000: Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agr.* 80(7), 825-860. DOI: 10.1002/(SICI)1097-0010(20000515)80:7<825::AID-JSFA598>3.0.CO;2-6
- DE JESÚS ORNELAS-PAZ, J., MARTÍNEZ-BURROLA, J.M., RUIZ-CRUZ, S., SANTANA-RODRÍGUEZ, V., IBARRA-JUNQUERA, V., OLIVAS, G.I., PÉREZ-MARTÍNEZ, J.D., 2010: Effect of cooking on the capsaicinoids and phenolic contents of Mexican peppers. *Food Chem.* 119(4), 1619-1625. DOI: 10.1016/j.foodchem.2009.09.054
- DEEPA, N., KAUR, C., GEORGE, B., SINGH, B., KAPOOR, H.C., 2007: Antioxidant constituents in some sweet pepper (*Capsicum annuum* L.) genotypes during maturity. *LWT-Food Science and Technology* 40(1), 121-129. DOI: 10.1016/j.lwt.2005.09.016
- DORJI, K., BEHBOUDIAN, M.H., ZEGBE-DOMINGUEZ, J.A., 2005: Water relations, growth, yield, and fruit quality of hot pepper under deficit irrigation and partial rootzone drying. *Sci. Hort.* 104(2), 137-149. DOI: 10.1016/j.scienta.2004.08.015
- ESTRADA, B., BERNAL, M.A., DÍAZ, J., POMAR, F., MERINO, F., 2000: Fruit development in *Capsicum annuum*: Changes in capsaicin, lignin, free phenolics, and peroxidase patterns. *J. Agric. Food Chem.* 48(12), 6234-6239. DOI: 10.1021/jf000190x
- GNAYFEED, M.H., DAOOD, H.G., BIACS, P.A., ALCARAZ, C.F., 2001: Content of bioactive compounds in pungent spice red pepper (paprika) as affected by ripening and genotype. *J. Sci. Food Agr.* 81(15), 1580-1585. DOI: 10.1002/jsfa.982
- HOWARD, L.R., TALCOTT, S.T., BRENES, C.H., VILLALON, B., 2000: Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J. Agric. Food Chem.* 48(5), 1713-1720. DOI: 10.1021/jf990916t
- JUÁREZ, L.Á.M., QUIJADA, D.M.M., SÁNCHEZ C.L.D.T., ÁGUILAR, G.A.G., MEZA, N.G., 2012: Antioxidant activity of peppers (*Capsicum annuum* L.) extracts and characterization of their phenolic constituents. *Inter-ciencia: Revista de ciencia y tecnología de América* 37(8), 588-593.
- KAUR, C., KAPOOR, H.C., 2001: Antioxidants in fruits and vegetables – the millennium's health. *Int. J. Food Sci. Tech.* 36(7), 703-725. DOI: 10.1111/j.1365-2621.2001.00513.x
- KHADI, B.M., GOUD, J.V., PATIL, V.B., 1987: Variation in ascorbic acid and mineral content in fruits of some varieties of chilli (*Capsicum annuum* L.). *Plant Foods Hum. Nutr.* 37(1), 9-15. DOI: 10.1007/BF01092295
- KIRSCHBAUM-TITZE, P., HIEPLER, C., MUELLER-SEITZ, E., PETZ, M., 2002: Pungency in paprika (*Capsicum annuum*). 1. Decrease of capsaicinoid content following cellular disruption. *J. Agric. Food Chem.* 50(5), 1260-1263. DOI: 10.1021/jf010527a
- KUMAR, O.A., TATA, S.S., 2009: Ascorbic acid contents in chili peppers (*Capsicum* L.). *Not. Sci. Biol.* 1(1), 50. DOI: 10.15835/nsb.1.1.3445
- LUDY, M.J., MATTES, R.D., 2011: The effects of hedonically acceptable red pepper doses on thermogenesis and appetite. *Physiol. Behav.* 102(3), 251-258. DOI: 10.1016/j.physbeh.2010.11.018
- MARTÍNEZ, S., CURROS, A., BERMÚDEZ, J., CARBALLO, J., FRANCO, I., 2007: The composition of Arnoia peppers (*Capsicum annuum* L.) at different

- stages of maturity. *Int. J. Food Sci. Nutr.* 58(2), 150-161.
DOI: 10.1080/09637480601154095
- MARTÍNEZ, S., LÓPEZ, M., GONZÁLEZ-RAURICH, M., BERNARDO ALVAREZ, A., 2005: The effects of ripening stage and processing systems on ascorbic acid content in sweet peppers (*Capsicum annuum* L.). *Int. J. Food Sci. Nutr.* 56(1), 45-51. DOI: 10.1080/09637480500081936
- MATERSKA, M., PERUCKA, I., 2005: Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *J. Agric. Food Chem.* 53(5), 1750-1756. DOI: 10.1021/jf035331k
- MATHUR, R., DANGI, R.S., DASS, S.C., MALHOTRA, R.C., 2000: The hottest chilli variety in India. *Curr. Sci.* 79(3), 287-288.
- MOHAMMADZADEH, S., SHARRIATPANAHI, M., HAMED, M., AMANZADEH, Y., EBRAHIMI, S.E.S., OSTAD, S.N., 2007: Antioxidant power of Iranian propolis extract. *Food Chem.* 103(3), 729-733.
DOI: 10.1016/j.foodchem.2006.09.014
- NIKLIŠ, N.D., SIOMOS, A.S., SFAKIOTAKIS, E.M., 2002: Ascorbic acid, soluble solids and dry matter content in sweet pepper fruit: change during ripening. *J. Vegetable Crop Prod.* 8(1), 41-51. DOI: 10.1300/J068v08n01_06
- OLIVEIRA, I., SOUSA, A., FERREIRA, I.C., BENTO, A., ESTEVINHO, L., PEREIRA, J.A., 2008: Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food Chem. Toxicol.* 46(7), 2326-2331. DOI: 10.1016/j.fct.2008.03.017
- RIGHETTO, A.M., NETTO, F.M., CARRARO, F., 2005: Chemical composition and antioxidant activity of juices from mature and immature acerola (*Malpighia emarginata* DC.). *Food Sci. Technol. Int.* 11(4), 315-321.
DOI: 10.1177/1082013205056785
- SCOVILLE, W.L., 1912: Note on capsicums. *J. Am. Pharm. Assoc.* 1(5), 453-454. DOI: 10.1002/jps.3080010520
- SIMONNE, A.H., SIMONNE, E.H., EITENMILLER, R.R., MILLS, H.A., GREEN, N.R., 1997: Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L.). *J. Food Comp. Anal.* 10(4), 299-311. DOI: 10.1006/jfca.1997.0544
- SINGLETON, V.L., ROSSI, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16(3), 144-158.
- TILAHUN, S., PARAMAGURU, P., RAJAMANI, K., 2013: Capsaicin and ascorbic acid variability in Chilli and Paprika cultivars as revealed by HPLC analysis. *J. Plant Breed. Genet.* 1(2), 85-89.
- USMAN, M.G., RAFIL, M.Y., ISMAIL, M.R., MALEK, M.A., LATIF, M.A., 2014: Capsaicin and dihydrocapsaicin determination in chili pepper genotypes using ultra-fast liquid chromatography. *Molecules* 19(5), 6474-6488.
DOI: 10.3390/molecules19056474
- VERA-GUZMÁN, A.M., CHÁVEZ-SERVIA, J.L., CARRILLO-RODRÍGUEZ, J.C., LÓPEZ, M.G., 2011: Phytochemical evaluation of wild and cultivated pepper (*Capsicum annuum* L. and *C. pubescens* Ruiz & Pav.) from Oaxaca, Mexico. *Chil. J. Agr. Res.* 71(4), 578.
DOI: 10.4067/S0718-58392011000400013
- ZHUANG, Y., CHEN, L., SUN, L., CAO, J., 2012: Bioactive characteristics and antioxidant activities of nine peppers. *J. Funct. Foods* 4(1), 331-338.
DOI: 10.1016/j.jff.2012.01.001

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