Doctoral Dissertation Theses

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Investigation of the bioactive components of hot pepper Vvarieties in relation to genotypes, growing season, and cultivation methods

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Approval of the School Principal Approval of the Supervisor

1. Background and objectives of the work

In my dissertation, I examined the nutritional components of four different types of hot peppers in relation to the cultivation method I chose, the genotype, and the weather conditions. The studied varieties were Bhut Jolokia (*Capsicum chinense* × *Capsicum frutescens*) chili pepper, Cserkó (Cap*sicum annuum* L. var. *cerasiforme*) cherry pepper, Szegedi 178 (*Capsicum annuum* L. var. *longum grossum*) spice pepper, and Rekord (*Capsicum annuum* L.) table pepper. These varieties are well-known and highly appreciated in Hungary, and they have been cultivated here for a long time.

Bioactive compounds found in plant-based foods generally have positive health effects on the human body. However, the content and type of these bioactive compounds can vary depending on genetic and environmental factors (Bae et al., 2014). Deepa et al. (2007) studied the vitamin C and capsaicinoid content in ripe and unripe peppers. Their results showed that ripe fruits had higher levels of ascorbic acid and capsaicinoids. According to Marín et al. (2004), however, flavonoid content decreases proportionally during pepper ripening. In the United States and Asia, the nutritional composition of different pepper types and their physiological and pharmacognostic effects are widely studied (Lantos, 2018). Hot peppers are a source of provitamin A, vitamins E and C, carotenoids, and phenolic compounds such as capsaicinoids, quercetin, and luteolin. These compounds exhibit antioxidant properties as well as other positive biological activities (Batiha et al., 2020). Different pepper varieties contain varying combinations of carotenoids, capsaicinoids, and capsinoids, which contribute to their distinct color and pungency (Yang et al., 2014).

Environmental conditions, agronomic traits, genetics, and other factors can alter the bioactive properties of foods (Jifon et al., 2012); however, only a few studies have reported on the effects of environmental factors and cultivation methods on the bioactive compound content of peppers. In Hungary, particularly in the Great Plain region, the main challenges in pepper and spice pepper cultivation are frequent drought, extreme weather, and difficulties with irrigation, all of which significantly affect yield and quality. In my research, I aimed to examine the nutritional components of four pepper varieties with different genetic traits under the given environmental conditions, in order to gain a clearer understanding of how these factors influence the presence of bioactive compounds in the fruits.

For the preparation of my thesis, I formulated the following objectives:

1. To investigate the bioactive components of four different hot pepper genotypes using modern HPLC methods.

2. To compare the amounts of the most important nutritional components (capsaicinoids, carotenoids, tocopherols, vitamin C) in the studied varieties.

3. To determine which factors influence the composition of these nutritional components (e.g., genotype, weather conditions, harvest time).

4. Additionally, to assess the effects of three factors (variety, growing season, harvest time) and their interactions on the dependent variables (capsaicinoids, carotenoids, tocopherols, vitamin C).

2. Materials and methods

Experimental conditions

The experiment was conducted at my residence in Pusztaföldvár (Békés County) under open-field conditions during the 2022 and 2023 growing seasons. GPS coordinates: 46.535054, 20.800754. Plot: EJYT5-8-17, Parcel No.: 117. No soil covering or mulching was applied during cultivation. Four hot pepper varieties with different pungency levels were selected for the experiment, and these were cultivated and studied in both years. The experiment was arranged in a randomized block design, with four replications per variety.

Seedlings were raised in a low-airspace, double-walled greenhouse equipped with heating. For sowing, propagation trays with hole dimensions of 37 × 37 × 40 mm were used, filled with Kekkilä DSM 3W peat substrate. Transplanting was carried out on May 13, 2022, and May 7, 2023. For each variety, 30 plants per replication were evaluated, totaling 480 plants. The row spacing was 90 cm, and the plant spacing within rows was 30 cm. Drip irrigation was used throughout the cultivation period.

Harvesting and Sample Preparation

Harvesting was carried out twice each year. In 2022, it took place on September 8 and 20, and in 2023 on August 30 and September 16. All varieties were harvested at full biological maturity. For each replication, at least 400 g of fruit per variety was collected for analysis. Each harvested sample was stored in a crate for one week in a well-ventilated area. Afterwards, the samples were halved and dried in the drying oven of the Faculty of Agriculture at the University of Szeged for 24 hours at 60 °C. This temperature was chosen as optimal for preserving color and other constituents. Completely dried samples were cooled to room temperature and vacuum-sealed. Samples were ground immediately before analytical measurements.

Determination of Carotenoids and Tocopherols

Fat-soluble carotenoids and tocopherols were extracted from ground pepper samples following a previously described protocol (Daood et al., 2014). Half a gram of well-homogenized sample was extracted with 50 mL of a 1:1:2 methanol-acetone-dichloromethane mixture by manual shaking at room temperature for 15 minutes. The mixture was then filtered through filter paper into a round-bottom flask. The solvent was evaporated to dryness at 40 °C using a vacuum evaporator (PRO-400, Vacuubrand, Germany). The residue was re-dissolved in 10 mL of a 55:18:24 methanol-2-propanol-acetonitrile mixture. Prior to HPLC analysis, the extract was further purified through a 0.22 µm hydrophobic PTFE syringe filter (13 mm).

Simultaneous HPLC determination of carotenoids and tocopherols was performed on a reversed-phase Nucleodur 100 column (3 µm, 240 × 4.6 mm) using gradient elution with 8% water in methanol (A) and 10:35:55 methanol-acetonitrile-2-propanol at a flow rate of 0.7 mL/min. DAD and fluorescence detectors were operated simultaneously for detection.

LC-DAD-MS/MS identification of carotenoids was carried out on a Waters Acquity I-class UPLC system coupled to a Waters Xevo TQ-XS MS/MS, following the detailed procedure of Duah et al. (2021a). Carotenoids were detected between 225 and 600 nm. Peaks were identified based on their spectral characteristics, mass spectra (protonated ion m/z values), retention times, and comparison with literature data (Schweiggert et al., 2007; Bonaccorsi et al., 2016). Cis-isomers were further identified by an additional absorption maximum between 340 and 362 nm and their Q-ratio values (Gupta et al., 2015; Schieber & Carle, 2005).

Tocopherols were detected with excitation at 325 nm and emission at 295 nm. Peaks were identified based on their spectral properties, mass spectra (protonated ion m/z values), and retention times in comparison with literature data (Schweiggert et al., 2007; Bonaccorsi et al., 2016).

Determination of capsaicinoids

For capsaicinoid analysis, 0.5 g of well-homogenized pepper powder from each sample was weighed into an Erlenmeyer flask, and 50 mL of HPLC-grade methanol was added. The flasks were placed on a mechanical rotary shaker at 300 rpm for 20 minutes, followed by 5 minutes of ultrasonic treatment using a water-bath ultrasonic device. The samples were then filtered through 0.45 µm Whatman No. 1 filter paper.

The filtered extracts were further cleaned by tenfold dilution with HPLC-grade methanol and passed through a Chromofil hydrophobic PTFE syringe filter before injection into the HPLC system. Capsaicinoids were separated on a Purospher Star C18 reversed-phase column (2.7 µm, 125 mm) using isocratic elution with a 48:52 water–acetonitrile mixture at a flow rate of 0.7 mL/min, following the method of Daood et al. (2015). Detection was performed using a fluorescence detector at Ex: 285 nm and Em: 320 nm. Peaks were identified based on retention times and previous HPLC-MS/MS protocols (Daood et al., 2015).

Determination of vitamin C

For vitamin C analysis, 0.5 g of pepper powder was weighed into a stoppered flask, and 30 mL of chilled 3% metaphosphoric acid was added. The mixture was shaken on a mechanical rotary shaker at 300 rpm for 20 minutes, transferred to centrifuge tubes, and centrifuged at 5,000 × g for 5 minutes. The supernatant was filtered through a 0.22 µm, 25 mm nylon syringe filter and injected into the HPLC system.

L-ascorbic acid (vitamin C) was separated from other organic acids and polar phenolic compounds on a Nautilus C18 aqua column (3 µm, 150 mm) using gradient elution with acetonitrile and 0.01 M potassium dihydrogen phosphate buffer at 0.7 mL/min. Detection was performed at 244 nm using a diode array detector (DAD). Identification of L-ascorbic acid was based on retention time and spectral characteristics compared to standards.

Data analysis

All statistical analyses were performed using R version 4.2.1 (RStudio Team, 2020). The effects of year, harvest, and variety on carotenoid, tocopherol, capsaicinoid, and vitamin C concentrations were evaluated using a general linear model (GLM). Treatment effects were accepted based on Wilks' lambda (λ). Homogeneity of the covariance matrix (homoscedasticity) was checked using Box's test. Normality of GLM residuals was tested with the Shapiro-Wilk test for all dependent variables, and the Kolmogorov-Smirnov test was also applied. Values shown above graphs represent mean ± standard deviation (SD). Effect size was estimated using partial eta-squared (η^2), indicating the probability of correctly identifying a significant effect; values range from 0 (independence) to 1.0 (deterministic relationship).

Cluster-based heatmaps were used to visually display metabolite profiles (carotenoids and xanthophyll compounds) using the 'pheatmap' package in R (Sfaxi et al., 2025). Input data were normalized column-wise using Z-score transformation:

$$Z = \frac{x-\mu}{\sigma} \quad (1)$$

where x represents the measured amount of each carotenoid, μ is the mean of that carotenoid across all varieties, and σ is the standard deviation. The color scale used in the heatmap illustrates deviations in concentration: negative Z-score values (below the mean) are shown in blue, while positive Z-score values (above the mean) are shown in red. Row and column clustering was performed using hierarchical clustering (Euclidean distance and "complete linkage" method), which organized the matrix based on similarities between samples and metabolites. The values in each cell represent the standardized (Z-score) values numerically.

3. Results and discussion

Environmental Factors (2022–2023)

Significant differences were observed between the two study years in terms of temperature and precipitation distribution. Statistically significant differences ($p < 0.05$) were found in average temperatures in June and September. In 2022, June and July recorded the lowest precipitation during the growing season, with average temperatures of 24–25 °C. In contrast, 2023 saw the opposite pattern, with a total of 419 mm of rainfall from May to August and average summer temperatures of 20–24 °C.

Precipitation in 2022 increased from 41 mm during the 15–20 days preceding the first harvest to 67 mm by the time of the second harvest, while temperatures decreased significantly. In 2023, precipitation dropped from 87 mm during the first harvest period to 18 mm during the second harvest period, while temperatures decreased slightly but remained above 22 °C.

Maximum temperatures in 2022, including the number of days exceeding 40 °C, as well as average temperatures, were considerably higher than in 2023, particularly three weeks before the first and second harvests. Longer periods of sunshine in 2022 also increased solar radiation intensity, which likely influenced the photosynthetic activity of the plants.

In August 2023 (87 mm), more rainfall occurred than in August 2022 (41 mm). Conversely, September 2022 (67 mm) was much wetter than September 2023 (18 mm), resulting in a reversed precipitation pattern during and immediately before the harvests.

Yield results (2022–2023)

Regarding weather conditions, the results suggest that the higher temperatures and lower precipitation in 2022 led to a slight increase in both fruit number per plant and fruit weight (g/fruit) compared to the 2023 harvest, which experienced lower temperatures and higher precipitation. An exception was the Bhut Jolokia variety, which produced a higher fruit number in 2023 despite the cooler temperatures and higher rainfall.

These findings indicate that harvestable yield is strongly influenced by both genetic and climatic conditions. Improvements in fruit number and weight during warmer growing seasons can be attributed to higher solar radiation intensity.

Carotenoids

Table 1 presents the changes in yellow carotenoids, showing the effects of variety, harvest time, and growing season on the dependent variables.

During the first harvest of 2022 (September 8), the Szegedi 178 variety exhibited significantly higher ($p < 0.05$) levels of free yellow xanthophylls (FYX: 370.73 µg/g) compared to Bhut Jolokia, Cserkó, and Rekord, according to Tukey post hoc tests. The yellow xanthophyll monoester (YXME) content was also highest in Szegedi 178 (633.67 µg/g), significantly exceeding the values of the other varieties, except for the first harvest of Rekord.

For total yellow carotenoids (TY), Szegedi 178 and Bhut Jolokia reached significantly higher levels (1780.38 µg/g and 1796.21 µg/g), whereas Cserkó and Rekord showed lower concentrations.

In the second harvest of 2022 (September 20), a marked increase in the yellow xanthophyll diester fraction (YXDE) was observed, especially in Bhut

Jolokia, where diester content doubled compared to monoesters as a result of weather changes preceding the second harvest.

In 2023, during the first harvest (August 30), the highest FYX values were recorded in Cserkó (130.18 μg/g) and Szegedi 178 (125.30 μg/g), although these were significantly lower than the corresponding 2022 values. For YXME, Szegedi 178 again stood out (566.23 μg/g). β-carotene values also decreased compared to the previous year.

During the second harvest in 2023 (September 16), FYX values were markedly lower than in 2022. Regarding YXDE, all varieties in 2023 showed lower diester content in both harvests compared to any harvest in 2022.

Overall, 2022 exhibited higher concentrations of all yellow carotenoids, while 2023 produced lower values, likely reflecting less favorable growing conditions. Szegedi 178 generally demonstrated superior nutritional quality, whereas Cserkó and Rekord had lower carotenoid content.

Table 1. Effect of variety, harvest time, and growing season on the concentration of yellow carotenoid groups (μg/g dry weight) in hot pepper powders.

Year	Harv.	Variety	FYX	YXME	YXDE	TY	AvP	βc
2022	1	BHJ	183,31 ± 1,50 AXa	459,86 ± 4,57 AXab	346,62 ± 8,57 AXa	1351,50 ± 18,02 AXab	425,10 ± 4,78 AXb	361,71 ± 4,03 AXb
		CS	234,27 ± 0,49 AXa	387,83 ± 1,16 AXa	226,28 ± 1,06 AXa	1013,99 ± 1,01 AXa	207,69 ± 0,58 AXa	165,61 ± 0,54 AXa
		R	211,33 ± 0,29 AXa	553,47 ± 0,65 AXbc	389,28 ± 0,70 AXa	1506,96 ± 1,51 AXb	438,83 ± 0,77 AXb	352,89 ± 0,68 AXb
		SZ178	370,73 ± 4,00 AXb	633,67 ± 5,83 AXc	308,21 ± 0,99 AXa	1605,60 ± 4,98 AXb	420,47 ± 4,57 AXb	292,99 ± 1,23 AXb
	2	BHJ	167,17 ± 0,62 AXa	443,72 ± 2,07 AXa	714,09 ± 4,41 AYb	1780,38 ± 9,50 AXa	529,26 ± 2,78 AXb	455,39 ± 2,47 AYc
		CS	226,19 ± 1,58 AXb	544,50 ± 6,14 AYab	393,93 ± 11,40 AXa	1446,05 ± 20,51 AXa	378,70 ± 6,22 AYa	281,43 ± 1,55 AYa
		R	159,99 ± 0,43 AYa	528,80 ± 1,16 AXa	415,84 ± 0,98 AXa	1479,55 ± 2,09 AXa	469,19 ± 0,66 AYab	374,92 ± 0,47 AYb
		SZ178	317,57 ± 0,88 AXc	683,04 ± 1,57 AXb	365,49 ± 0,98 AYa	1796,21 ± 3,28 AYa	509,71 ± 1,32 AXab	430,11 ± 1,20 AYbc
2023	1	BHJ	118,89 ± 0,49 BXab	442,35 ± 1,67 AXa	150,31 ± 0,34 AXb	859,35 ± 2,70 BXa	400,70 ± 1,33 AXab	132,88 ± 0,62 BXa
		CS	130,18 ± 0,49 BXb	543,66 ± 1,03 BXbc	86,32 ± 0,29 BXa	888,59 ± 2,10 BXa	435,34 ± 1,15 BXab	116,45 ± 0,44 BXa
		R	102,66 ± 0,24 BXa	452,46 ± 1,75 BXab	165,11 ± 0,71 BXb	868,40 ± 2,93 BXa	398,11 ± 1,56 AXa	125,88 ± 0,46 BXa
		SZ178	125,30 ± 0,76 BXb	566,23 ± 3,99 AXc	106,51 ± 1,03 BXa	950,82 ± 6,68 BXa	482,46 ± 3,19 AXb	139,68 ± 0,85 BXa
	2	BHJ	125,61 ± 0,27 BXb	277,05 ± 0,57 BYb	204,07 ± 0,37 BYc	769,33 ± 1,37 BYb	218,63 ± 0,37 BYc	162,59 ± 0,24 BYc
		CS	119,41 ± 0,29 BXab	230,34 ± 0,74 BYa	97,96 ± 0,47 BXa	521,80 ± 1,62 BYa	107,47 ± 0,48 BYa	74,09 ± 0,32 BYa
		R	111,32 ± 0,28 BXa	270,90 ± 0,91 BYab	264,54 ± 0,63 BYd	790,79 ± 2,06 BXb	220,90 ± 0,51 BYc	144,03 ± 0,34 BYc
		SZ178	145,77 ± 0,41 BXc	336,11 ± 1,51 BYc	163,92 ± 1,19 BYb	768,01 ± 3,80 BXb	183,84 ± 1,14 BYb	122,21 ± 0,81 BXb

The table uses the following abbreviations: free yellow xanthophylls (FYX), yellow xanthophyll monoester (YXME), yellow xanthophyll diester (YXDE), total yellow carotenoids (TY), vitamin A precursors (AvP), and β-carotene (βc). All values represent the mean ± SD of four independent replicates. Different letters indicate significant differences: uppercase letters (A, B) denote differences between years and harvests (X, Y), while lowercase letters indicate differences between varieties (Tukey's HSD test, $p < 0.05$).

Table 2 shows the changes in red carotenoids, illustrating the effects of variety, harvest time, and growing season on the dependent variables. The influence of the three main factors was assessed using Tukey post hoc tests.

During the first harvest of 2022 (September 8), Szegedi 178 (454.55 µg/g) and Cserkó (369.82 µg/g) exhibited the highest levels of free red xanthophylls (FRX), significantly exceeding those of Bhut Jolokia (112.27 µg/g) and Rekord (159.80 µg/g). For red xanthophyll monoesters (RXME), Szegedi 178 again showed the highest content. Total red carotenoids (TR) were also highest in Szegedi 178 (3734.95 µg/g), whereas Rekord (2417.79 µg/g) and Cserkó (2033.31 µg/g) showed lower levels, with Bhut Jolokia being the lowest (1871.70 µg/g). Considering total visible carotenoids (TCv), Szegedi 178 reached 5340.55 µg/g, significantly higher than the other varieties in both harvests.

During the second harvest of 2022 (September 20), concentrations of all red carotenoids, including FRX, RXME, and RXDE, decreased in all varieties compared to the first harvest. This indicates that the weather conditions preceding the second harvest were unfavorable for red xanthophyll biosynthesis, particularly in Rekord (60.24 µg/g) and Cserkó (278.23 µg/g). Szegedi 178 still showed the highest value (419.85 µg/g), although it decreased compared to the first harvest. TR was highest in Szegedi 178 (3597.97 µg/g), while significant decreases were observed in Rekord (2159.60 µg/g) and Bhut Jolokia (1207.93 µg/g).

In the 2023 season, red carotenoid contents were generally significantly lower ($p < 0.01$) than in 2022, likely due to higher precipitation during the growing season. Another difference between the two years was the effect of harvest timing on red carotenoids: the second harvest often led to a slight or significant increase in carotenoid content for most varieties.

During the first harvest of 2023 (August 30), RXME was highest in Cserkó (394.44 µg/g) and lowest in Rekord (265.99 µg/g). RXDE was also highest in Szegedi 178 (627.03 µg/g), while Rekord (503.09 µg/g) and Bhut Jolokia (495.54 µg/g) showed lower values. TR values were significantly higher in Cserkó (1132.71 µg/g) and Szegedi 178 (1112.56 µg/g) compared to Rekord (830.94 µg/g) and Bhut Jolokia (904.12 µg/g), with similar trends observed for TCv.

During the second harvest of 2023 (September 16), FRX further decreased compared to the first harvest, except in Bhut Jolokia. Szegedi 178 maintained the highest value (175.78 µg/g). For RXME, Bhut Jolokia showed higher content (360.24 µg/g), while Rekord remained the lowest (268.11 µg/g). RXDE and TR were also highest in Szegedi 178 (785.60 µg/g and 1467.75 µg/g, respectively).

Table 2. Effect of variety, harvest time, and growing season on the concentration of red carotenoid groups (µg/g dry weight) in hot pepper powders.

Year	Harv.	Variety	FRX	RXME	RXDE	TR	TcV
2022	1	BHJ	112,27 ± 0,79 AXa	428,33 ± 3,41 AXa	1331,10 ± 19,08 AXab	1871,70 ± 23,18 AXa	3223,20 ± 41,15 AXa
		CS	369,82 ± 0,97 AXb	634,25 ± 1,00 AXb	1029,24 ± 2,39 AXa	2033,31 ± 2,51 AXa	3047,30 ± 2,16 AXa
		R	159,80 ± 0,49 AXa	577,14 ± 0,95 AXb	1680,86 ± 1,86 AXb	2417,79 ± 3,03 AXa	3924,75 ± 4,23 AXa
		SZ178	454,55 ± 4,33 AXb	1097,39 ± 4,38 AXc	2183,01 ± 8,40 AXc	3734,95 ± 14,73 AXb	5340,55 ± 19,56 AXb
	2	BHJ	93,53 ± 0,26 AXa	336,97 ± 1,85 AXa	777,44 ± 5,31 AYa	1207,93 ± 7,26 AYa	2988,31 ± 16,67 AXa
		CS	278,23 ± 0,79 AYb	582,99 ± 3,46 AXb	1096,92 ± 12,90 AXb	1958,13 ± 16,49 AXb	3404,18 ± 10,75 AYab
		R	60,24 ± 0,39 AYa	450,73 ± 2,19 AYa	1648,62 ± 4,08 AXc	2159,60 ± 6,22 AYb	3639,15 ± 8,15 AYb
		SZ178	419,85 ± 1,49 AXc	1075,78 ± 3,45 AXc	2102,33 ± 3,44 AXd	3597,97 ± 7,50 AXc	5394,18 ± 9,58 AXc
2023	1	BHJ	100,83 ± 0,41 AXab	307,74 ± 1,61 BXab	495,54 ± 1,82 BXa	904,12 ± 3,79 BXa	1629,47 ± 5,92 BXab
		CS	171,32 ± 1,05 BXc	394,44 ± 0,88 BXc	566,95 ± 1,36 BXab	1132,71 ± 2,98 BXb	1896,82 ± 4,90 BXb
		R	61,86 ± 0,10 BXa	265,99 ± 1,03 BXa	503,09 ± 1,89 BXa	830,94 ± 2,89 BXa	1522,66 ± 5,40 BXa
		SZ178	132,37 ± 0,82 BXbc	353,15 ± 1,82 BXbc	627,03 ± 3,86 BXb	1112,56 ± 6,25 BXb	1931,07 ± 12,11 BXb
	2	BHJ	107,20 ± 0,30 BXb	360,24 ± 0,97 AYb	580,55 ± 1,20 BYb	1047,99 ± 2,42 AYb	1949,27 ± 4,39 BYb
		CS	166,50 ± 0,32 BXb	357,34 ± 1,01 BYb	463,50 ± 1,53 BYa	987,34 ± 2,68 BYab	1617,24 ± 4,46 BYa
		R	60,12 ± 0,30 AXa	268,11 ± 1,01 BXa	558,29 ± 1,40 BXb	886,52 ± 2,68 BXa	1804,51 ± 5,24 BYab
		SZ178	175,78 ± 0,33 BYc	506,37 ± 1,72 BYc	785,60 ± 3,33 BYc	1467,75 ± 5,15 BYc	2463,34 ± 9,62 BYc

The table uses the following abbreviations: free red xanthophylls (FRX), red xanthophyll monoester (RXME), red xanthophyll diester (RXDE), total red carotenoids (TR), and total visible carotenoids (TCv). All values represent the mean ± SD of four independent replicates. Different letters indicate significant differences: uppercase letters (A, B) denote differences between years and harvests (X, Y), while lowercase letters indicate differences between varieties.

Differences between growing seasons and the effect of harvest timing on color quality and stability are more clearly observed using the ratio of red to yellow carotenoids (R/Y), as shown in **Figure 1**. In 2022, the R/Y ratio showed a decreasing trend toward the second harvest in all varieties, likely due to lower nighttime temperatures preceding harvest. In contrast, in 2023, the R/Y ratio increased in all varieties at the second harvest, when precipitation was significantly lower. The highest R/Y ratio was recorded in Szegedi 178 powder during the first harvest of 2022 and, along with Cserkó, during the second harvest of 2023. High R/Y ratios are important for evaluating the quality, market value, and expected storage stability of pepper-based products.

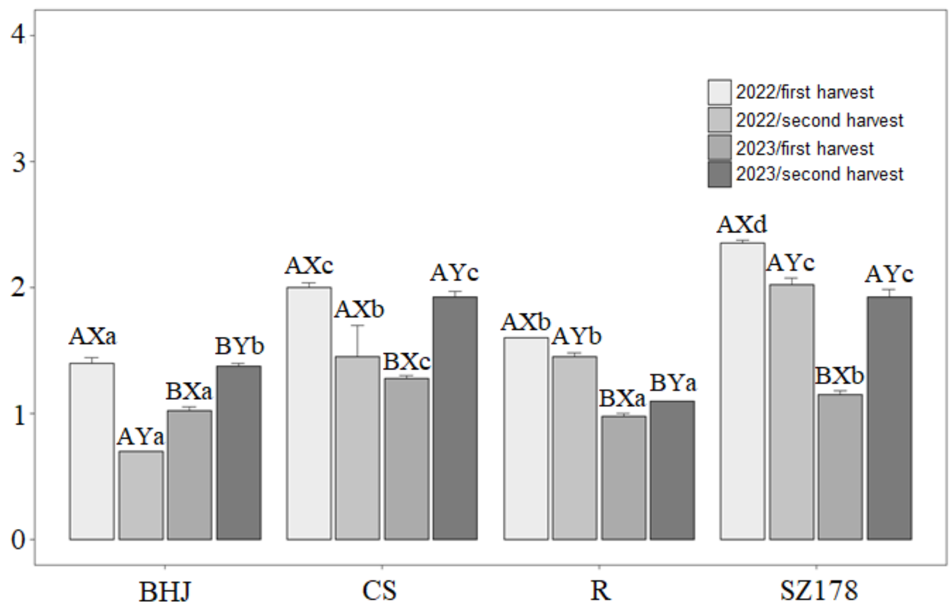

Figure 1. Ratio of red to yellow carotenoids (R/Y). Uppercase letters (A, B) indicate differences between varieties across years and harvests (X, Y), while lowercase letters indicate differences between varieties within the same year.

Figure 2. show the Z-score data of the analyzed carotenoids. The rows show the different pepper varieties (or the variety × year × harvest combinations), and the columns indicate the individual carotenoid components. The different colors reflect the Z-score values, where a red or reddish cell indicates that the given component (column) shows an above-average amount in the given pepper variety (row) in the specific year and harvest time. Blue indicates that the component amount is below average. White or very light colors indicate values close to the average.

From the heatmap, it can be seen that Szegedi 178 exceeded the other varieties, years, and harvest results in several carotenoid components in the first and second harvests of 2022. In this case, RXDE, FRX, RXME, FYX, and YXME amounts were well above average. In Bhut Jolokia and Rekord varieties, a larger deviation can also be observed in the first and second harvests of 2022, but here the positive deviation from the averages was in YXDE, YXME, βc, and AvP content. The largest negative deviation for all varieties was observed in the second harvest of 2023, mostly for yellow carotenoids. Overall, it can be stated that among the investigated components, the highest above-average carotenoid amounts were found in the first and second harvests of 2022. In 2023, they were mostly below average, especially in the second harvest.

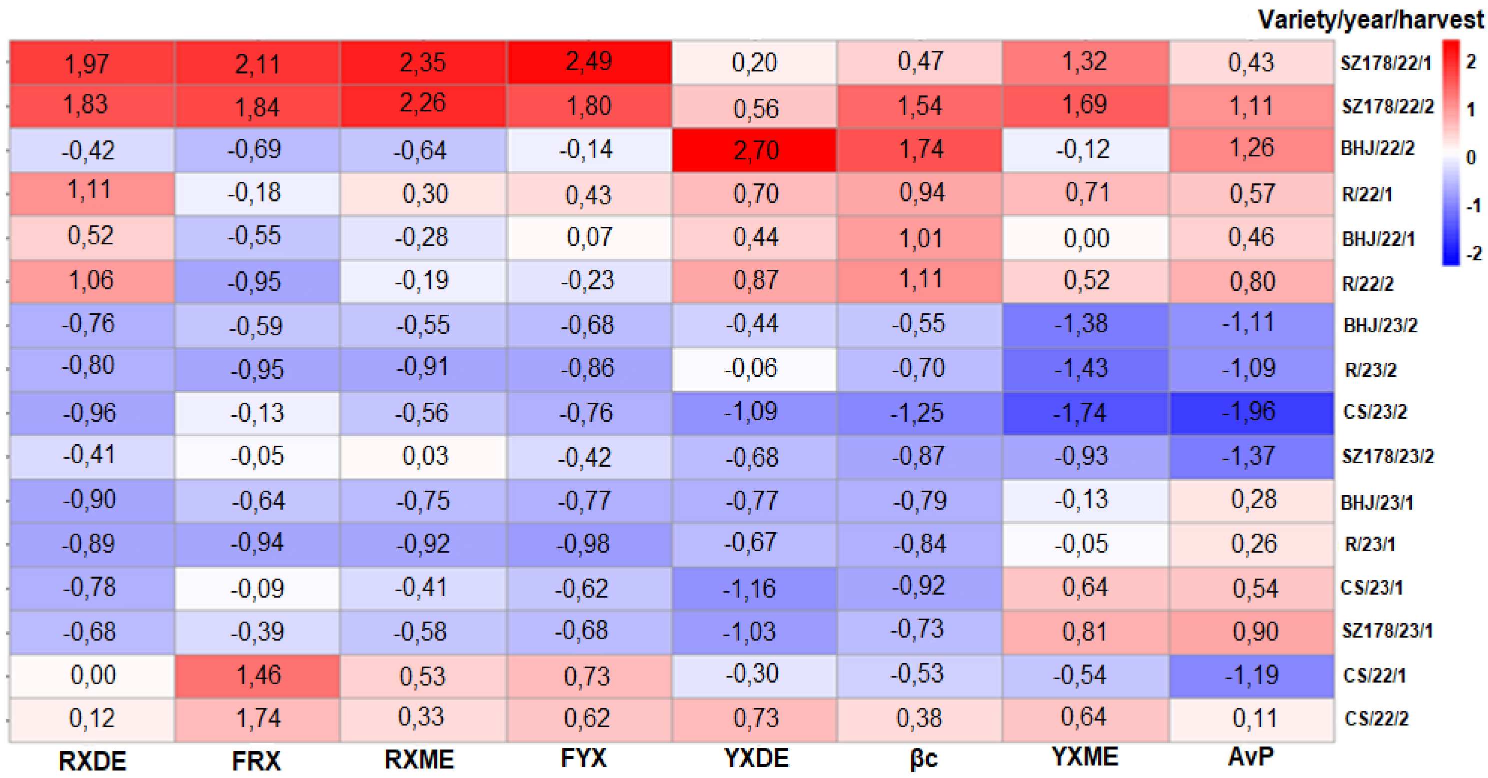

Figure 2. Heatmap of pepper varieties based on carotenoid components (Z-score normalized). Red indicates a high Z-score, i.e., a positive deviation from the mean, while blue indicates a negative deviation. Values close to 0 (white) indicate that the component concentration is near the average, with no significant differences between varieties. The lines represent hierarchical clustering, grouping varieties and carotenoid components based on their similarity.

Tocopherols

The reversed-phase HPLC protocol used for the separation and quantification of vitamin E compounds allowed the separation of the main tocopherols and their derivatives, except for dehydrogenated tocotrienols, which are scarcely present in paprika **(Table 3).**

In the first harvest of 2022, Szegedi 178 showed the highest values for all tocopherols, particularly α-Toc (601.08 µg/g) and total tocopherols (Total) (938.50 µg/g). In the second harvest, apart from Rekord α-TocHQ and Szegedi 178 α-TocAC, no significant differences were observed compared to the first harvest for the other varieties.

In the first harvest of 2023, α-Toc and Total contents were higher compared to 2022 in most varieties. For example, Bhut Jolokia showed a significant increase in α-Toc (752.12 µg/g) and Total (997.00 µg/g). The Cserkó variety had an exceptionally high α-TocHQ value (260.77 µg/g). During the second harvest, a decrease in α-TocHQ was observed, which was significant only in Bhut Jolokia and Rekord. However, Szegedi 178 continued to show outstanding α-Toc (836.01 µg/g) and Total (1205.98 µg/g) tocopherol content.

3Table 3. Effect of variety, harvest time, and growing season on tocopherol concentrations (µg/g dry weight) in hot pepper powders.

Year	Harv.	Variety	α-TocHQ	α-Toc	α-TocAC	α-TocES	Total
2022	1	BHJ	28,19 ± 5,15 AXa	515,11 ± 71,68 AXbc	34,03 ± 6,53 AXa	26,09 ± 4,88 AXa	603,43 ± 87,39 AXb
		CS	167,36 ± 9,73 AXd	433,41 ± 18,89 AXb	83,47 ± 5,66 AXb	63,64 ± 3,36 AXb	747,88 ± 28,39 AXb
		R	35,20 ± 5,78 AXb	129,62 ± 8,33 AXa	60,81 ± 2,59 AXb	54,52 ± 1,69 AXb	280,15 ± 12,32 AXa
		SZ178	90,25 ± 17,33 AXc	601,08 ± 60,45 AXc	151,70 ± 20,42 AXc	95,49 ± 18,39 AXc	938,50 ± 110,50 AXc
	2	BHJ	27,87 ± 1,36 AXa	445,67 ± 38,58 AXbc	37,75 ± 2,63 AXa	21,78 ± 1,58 AXa	533,07 ± 43,30 AXab
		CS	114,54 ± 44,39 AXb	337,37 ± 126,84 AXb	91,94 ± 36,49 AXb	52,08 ± 19,56 AXb	595,92 ± 227,03 AXb
		R	51,83 ± 8,12 AYa	136,12 ± 16,89 AXa	58,97 ± 3,07 AXab	54,83 ± 4,19 AXb	301,75 ± 23,81 AXa
		SZ178	100,83 ± 7,31 AXb	580,69 ± 66,14 AXc	198,43 ± 10,82 AYc	101,57 ± 13,54 AXc	981,53 ± 79,17 AXc
2023	1	BHJ	43,38 ± 5,70 BXa	752,12 ± 89,08 BXa	139,18 ± 35,49 BXb	62,32 ± 13,61 BXa	997,00 ± 142,65 BXa
		CS	260,77 ± 32,37 BXc	676,01 ± 57,00 BXa	**55,10** ± 8,28 BXa	76,36 ± 4,64 BXa	1068,23 ± 66,42 BXa
		R	115,56 ± 26,77 BXb	402,61 ± 303,15 AXa	96,88 ± 28,52 BXab	62,29 ± 5,40 BXa	677,34 ± 356,16 AXa
		SZ178	151,71 ± 27,81 BXb	675,23 ± 292,33 AXa	100,54 ± 21,20 BXab	62,08 ± 6,35 BXa	989,56 ± 335,54 BXa
	2	BHJ	31,54 ± 1,99 BYa	765,56 ± 38,52 BXc	175,74 ± 17,79 BXb	55,95 ± 6,33 BXa	1028,79 ± 63,67 BXb
		CS	255,25 ± 22,81 BXd	550,35 ± 28,07 BYb	92,69 ± 9,66 AYa	68,52 ± 4,05 AYb	966,81 ± 55,19 BXb
		R	56,65 ± 10,37 AYb	206,73 ± 22,25 BXa	87,11 ± 6,37 BXa	50,21 ± 3,85 AYa	400,70 ± 39,60 BXa
		SZ178	119,78 ± 7,16 BXc	836,01 ± 23,80 BXd	168,36 ± 6,68 BYb	81,83 ± 5,28 BYc	1205,98 ± 18,89 BXc

The labels in the figure represent the following: α-TocHQ: α-tocopherol hydroquinone; α-Toc: α-tocopherol; α-TocAC: α-tocopherol acetate; α-TocES: α-tocopherol succinate; Total: total identified tocopherols.

Capsaicinoids

In 2022, capsaicinoid content differed significantly between varieties (**Table 4**); however, the harvest time was rarely a statistically significant factor. During the first harvest, CAP (capsaicin) content was highest in Bhut Jolokia (1586.36 µg/g) and lowest in Rekord (514.24 µg/g). DC (dihydrocapsaicin) was similarly high in Szegedi 178 (1263.53 µg/g) and Cserkó (1133.67 µg/g), while the lowest value was recorded in Bhut Jolokia (640.13 µg/g). In the second harvest, NDC content increased in all varieties, although this was not statistically significant. DC values increased in all varieties, particularly in Szegedi 178 (1448.39 µg/g) and Cserkó (1171.11 µg/g), as well as in Rekord, which showed the largest proportional increase (from 522.76 µg/g to 744.30 µg/g), though these changes were not statistically significant.

In 2023, the highest NDC value during the first harvest was observed in Szegedi 178 (208.28 µg/g), but it decreased considerably by the second harvest (47.26 µg/g). CAP concentration was most pronounced in Szegedi 178 (1233.90 µg/g), further increasing to 1430.46 µg/g in the second harvest.

These results indicate that, based on statistical tests, significant differences were observed between varieties and harvest times ($p < 0.05$), suggesting that both genetic background and harvest timing have a substantial effect on the measured parameters.

Table 4. Capsaicinoid content (µg/g DW = µg/g dry weight) as affected by variety, harvest time, and growing season in 2022 and 2023.

Év	Betak.	Fajta	NDC	CAP	DC	HCAP	HDC-2
2022	1	BHJ	36,11 ± 5,36 AXa	1586,36 ± 123,35 AXc	640,13 ± 85,04 AXa	35,04 ± 4,86 AXb	5,27 ± 0,52 AXa
		CS	246,31 ± 25,12 AXd	1339,94 ± 73,36 AXbc	1133,67 ± 83,22 AXb	9,87 ± 0,90 AXa	92,25 ± 6,84 AXd
		R	82,39 ± 3,50 AXb	514,24 ± 38,25 AXa	522,76 ± 26,40 AXc	10,37 ± 0,81 AXa	29,41 ± 1,26 AXb
		SZ178	195,02, ± 22,33 AXc	1200,74 ± 124,39 AXb	1263,53 ± 123,30 AXb	30,33 ± 4,12 AXb	65,38 ± 6,30 AXc
	2	BHJ	37,88 ± 1,10 AXa	1734,01 ± 44,41 AXc	646,98 ± 7,70 AXa	10,05 ± 1,08 AYa	10,16 ± 0,37 BXa
		CS	268,84 ± 16,28 AXc	1319,94 ± 145,89 AXb	1171,11 ± 103,56 AXb	8,28 ± 0,70 AYa	104,98 ± 6,78 AXd
		R	129,41 ± 21,22 AXb	771,99 ± 147,49 AXab	744,30 ± 125,36 AXab	14,15 ± 2,10 AXa	43,10 ± 5,67 BYb
		SZ178	239,06 ± 32,32 AXc	1401,93 ± 132,62 AXb	1448,39 ± 161,53 AXc	37,91 ± 5,04 AXb	81,01 ± 10,60 AXc
2023	1	BHJ	73,04 ± 9,72 BXa	919,09 ± 26,91 BXb	854,83 ± 38,27 BXb	25,58 ± 11,99 AXb	18,98 ± 3,23 BXa
		CS	38,72 ± 3,58 BXa	246,03 ± 38,21 BXa	**289,00** ± 32,97 BXa	5,56 ± 1,65 BXa	22,76 ± 1,35 BXa
		R	48,61 ± 9,55 BXa	241,38 ± 38,18 BXa	337,19 ± 51,57 BXa	12,01 ± 1,38 AXab	29,57 ± 4,49 BXa
		SZ178	208,28 ± 31,47 AXb	1233,90 ± 218,18 AXc	1417,39 ± 224,81 AXc	63,04 ± 12,52 BXc	81,22 ± 11,50 BXb
	2	BHJ	51,03 ± 23,31 AXa	786,38 ± 490,60 BXb	775,94 ± 72,51 BXa	17,23 ± 1,78 AXa	22,90 ± 1,56 AXa
		CS	38,28 ± 5,49 BXa	202,29 ± 59,04 BYa	267,50 ± 63,36 BXa	4,08 ± 0,92 BYa	20,59 ± 2,05 BYab
		R	81,18 ± 10,84 BYa	558,50 ± 154,48 AYab	664,62 ± 98,34 AYa	13,66 ± 1,54 AXa	39,77 ± 4,62 AYab
		SZ178	47,26 ± 54,49 BYa	1430,46 ± 236,65 BYab	1425,24 ± 218,95 AXc	15,18 ± 1,78 BYb	76,42 ± 9,46 BYb

Uppercase letters (AB) indicate differences between varieties across years and between harvests (XY). Lowercase letters indicate differences between varieties. Values sharing the same letter within a column are not significantly different (Tukey HSD test, $p < 0.05$). Abbreviations are as follows: NDC: nordihydrocapsaicin, CAP: capsaicin, DC: dihydrocapsaicin, HCAP: homocapsaicin, HDC-2: homodihydrocapsaicin-2.

In **Table 5**, the CAP/DC (capsaicin/dihydrocapsaicin) ratio is shown, where significant differences were observed between varieties and harvest times in 2022 and 2023. These two components make up the largest portion of the total capsaicinoid content in hot peppers. During the first harvest in 2022, Bhut Jolokia showed the highest value (2.48), which decreased significantly in 2023 (1.08). A decrease was also observed in most varieties during the second harvest. Overall, Bhut Jolokia exhibited the highest CAP/DC ratio in both years and at both harvest times. In 2023, the ratio decreased for all varieties compared to the previous year, except for Szegedi 178, where a slight increase was observed. Statistical analysis of the CAP/DC ratios revealed that significant differences were found only between varieties, not between years or harvest times.

Table 5. Changes in the capsaicin to dihydrocapsaicin (CAP/DC) ratio.

Year	Harvest	Variety	CAP/DC
2022	September 8	Bhut Jolokia	2,48 ± 0,33 ^{AXa}
		Cserkó	1,18 ± 0,06 ^{AXb}
		Rekord	0,98 ± 0,07 ^{AXc}
		Szegedi 178	0,95 ± 0,12 ^{AXc}
	September 20	Bhut Jolokia	2,03 ± 0,05 ^{AXa}
		Cserkó	1,13 ± 0,10 ^{AXb}
		Rekord	1,04 ± 0,20 ^{AXb}
		Szegedi 178	0,97 ± 0,16 ^{AXb}
2023	August 30	Bhut Jolokia	1,08 ± 0,03 ^{AXa}
		Cserkó	0,58 ± 0,04 ^{AXb}
		Rekord	0,72 ± 0,11 ^{AXc}
		Szegedi 178	0,87 ± 0,12 ^{AXc}
	September 16	Bhut Jolokia	1,01 ± 0,08 ^{AXa}
		Cserkó	0,76 ± 0,13 ^{AXcb}
		Rekord	0,84 ± 0,10 ^{AXb}
		Szegedi 178	1,01 ± 0,11 ^{AXa}

In **Figure 3**, it can be clearly observed that some varieties showed predominantly negative Z-scores (blue color) for most capsaicinoids, meaning that the content of individual capsaicinoids was lower than the average. This was especially evident for CAP and DC in the first and second harvests of 2023 for the Rekord and Cserkó varieties. In contrast, varieties harvested in 2022 showed higher values (red color) for several capsaicinoids in both harvests, indicating that these pepper samples had a stronger capsaicinoid profile. In the Szegedi 178 variety, most of the investigated capsaicinoid contents were above average.

When comparing individual varieties, it is evident that different years and harvest times for the same variety also caused variations in the content of specific capsaicinoids. This suggests that environmental factors (in this case, weather and harvest timing) significantly influenced the levels of compounds responsible for pungency even within the same varieties. The results help highlight how stable or variable a particular capsaicinoid is under different conditions across different varieties.

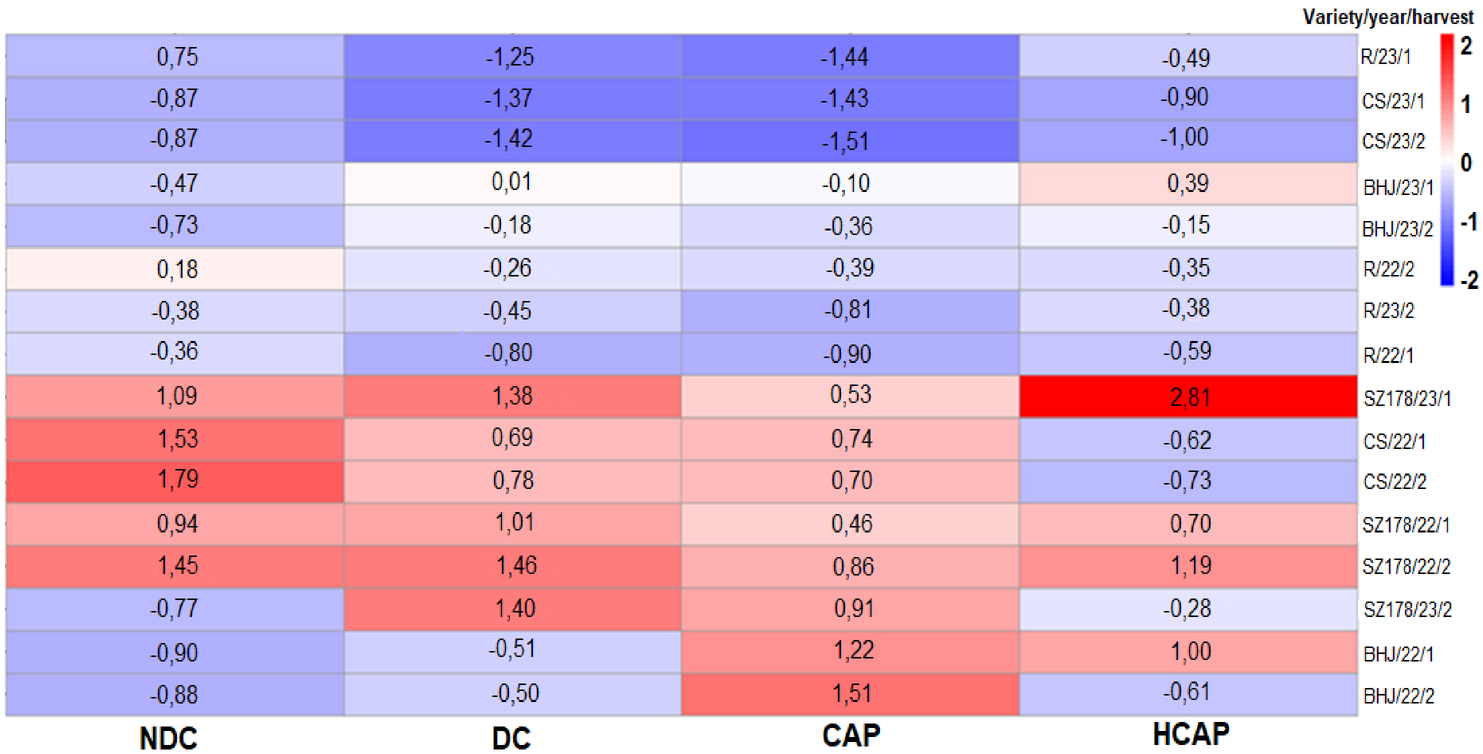

Figure 3. Heatmap of pepper varieties based on capsaicinoid components (Z-score normalized). The red color indicates a high Z-score, i.e., a positive deviation from the mean, while the blue color indicates a negative deviation. Values close to 0 (white) show that the component concentration is near the average, with no significant difference between varieties. The lines represent hierarchical clustering, grouping the varieties and capsaicinoid components based on their similarity.

Vitamin C

Regarding vitamin C content, the evaluated genotypes showed significant differences across years and harvest times (**Figure 4**). In 2022, during the first harvest, the highest vitamin C content (4060.67 µg/g) was measured in the Bhut Jolokia variety, while the lowest (1232.46 µg/g) was observed in the Cserkó variety. During the second harvest, an increase in vitamin C content was observed in all varieties.

In 2023, during the first harvest, the Bhut Jolokia variety exhibited a significant increase compared to the previous year, showing the highest vitamin C content (5309.00 µg/g) among the varieties. Under the same conditions, the Cserkó variety had 2188.13 µg/g, which significantly decreased compared to the second harvest of the previous year. During the second harvest of 2023, a decrease in vitamin C content was observed in all varieties except for Rekord, which showed a significantly higher content compared to previous harvests.

Examining the effect of meteorological conditions at harvest time, it was found that all genotypes showed a significant increase in vitamin C concentration as precipitation also significantly increased ($p < 0.01$) during the second harvest of the first growing season. This increase was particularly notable in the Cserkó variety, where vitamin C concentration nearly tripled, reaching the second-highest level after Bhut Jolokia.

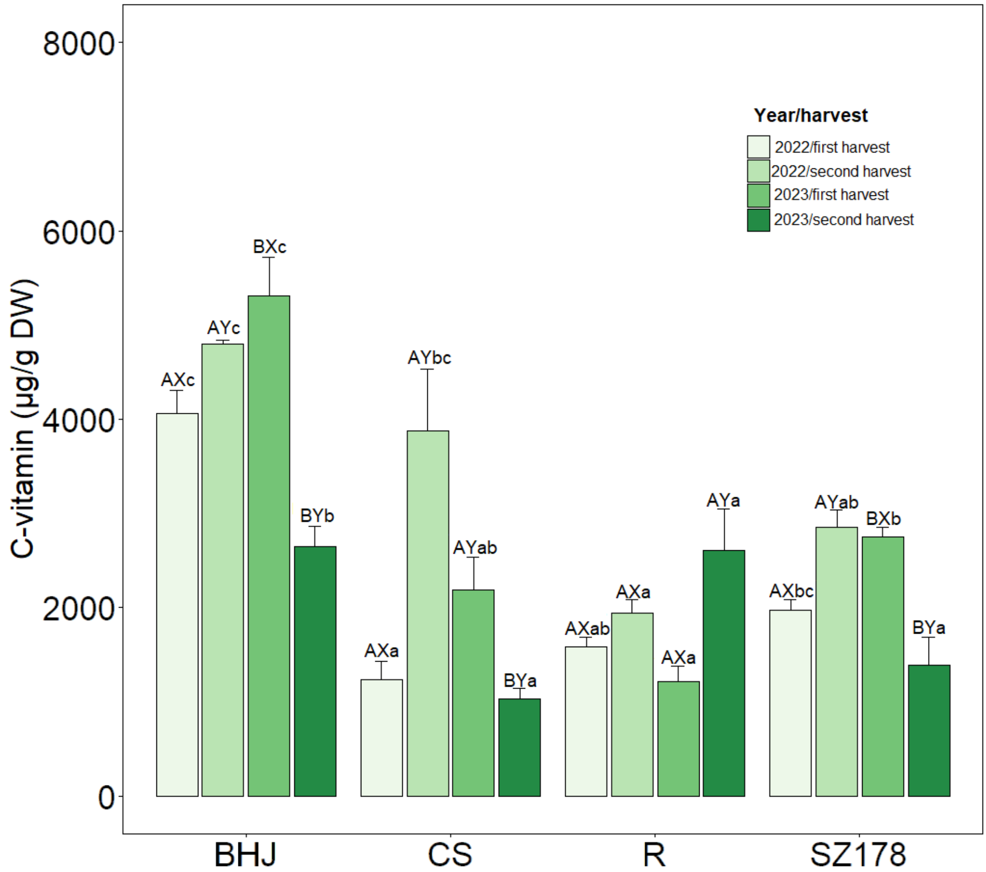

Figure 4. Changes in vitamin C content in 2022 and 2023 at two different harvest times. Capital letters (AB) indicate differences within varieties between years and between harvests (XY); lowercase letters indicate differences among different varieties within the same year. Means within a column sharing the same letter do not differ significantly (Tukey HSD test, $p < 0.05$).

4. Conclusions and recommendations

Significant differences were observed between the 2022 and 2023 seasons for total yellow carotenoids. In 2022, the β-carotene content of fruits at the second harvest was higher than that measured at the first harvest, likely due to abiotic factors such as air temperature, precipitation, and periods of sunshine, which aligns with the findings of Duah et al. (2021b). The response of β-carotene to abiotic factors was similar across all cultivars studied, with Szegedi 178 highlighted for showing a 46% increase at the second harvest compared to the first in 2022.

A similar trend was observed for all A-vitamin precursors. In contrast, during the 2023 season, an opposite trend was noted for A-vitamin precursor concentrations: higher values ($p < 0.01$) were recorded at the first harvest than at the second, supporting the idea that the biosynthesis of major yellow carotenoids is strongly influenced by the weather conditions prior to harvest. The largest decrease in A-vitamin precursors (76%) was observed in Cserkó, followed by Szegedi 178 with a 54% decrease, indicating their high sensitivity to environmental conditions.

Differences among cultivars were also evident in the esterification of xanthophylls with fatty acids. Yellow xanthophylls were predominantly present as monoesters. Variations between cultivars are likely due to the effect of pre-harvest weather on the esterification process.

At the second harvest of the 2022 season, the highest A-provitamin and β-carotene content was observed in Bhut Jolokia, followed by Szegedi 178. Consuming 10 g/day of Bhut Jolokia powder corresponds to 380 µg retinol activity equivalents (RAE).

Similar to yellow xanthophylls, red carotenoids were also present as fatty acid esters, with diesters predominating. These compounds are responsible for the red color and its stability in both fresh and processed red pepper products, consistent with the findings of Jang et al. (2022), who reported that the total carotenoid content of red peppers is closely correlated with red xanthophyll levels.

It can be concluded that cooler pre-harvest temperatures and high precipitation negatively affected the biosynthetic pathway of red pigments (Duah et al., 2021b). The major groups of red carotenoids tend to vary in parallel with total visible carotenoids, as red xanthophylls dominate in pepper samples. Since the levels of red xanthophyll diesters and monoesters mirrored the total red carotenoid content, it can be expected that products made from Szegedi 178 and Rekord cultivars would have higher shelf-life and marketability than products from other cultivars. Differences in diester ratios between cultivars may alter expectations regarding storage stability of products from different pepper varieties. It was observed that lower precipitation corresponded to higher red pigment ratios, a finding similar to those of Duah et al. (2021a) and Ráth et al. (2020). The 2022 season's abiotic factors, such as temperature and precipitation, appeared ideal for the development of outstanding traits (e.g., intense red color and high nutritional value).

Similar to red carotenoids, E-vitamin positively responded ($p < 0.05$) to decreased precipitation in the second harvest of Szegedi 178 in 2023, likely due to a shared biochemical pathway for carotenoid and tocopherol biosynthesis. This trend applied to all E-vitamin components. Interestingly, in 2022, when precipitation was higher, there were no significant differences between harvests in E-vitamin content among cultivars, except for Cserkó, where vitamin levels significantly decreased ($p < 0.05$) at the second harvest. These results suggest that the degree of E-vitamin oxidation is independent of its biosynthesis and is more influenced by genetic factors regulating redox pathways; responses to abiotic factors differed by genotype.

In 2023, the pre-harvest period was characterized by relatively higher temperatures (29 °C) and extremely low precipitation (18 mm) compared to 2022. The response of capsaicinoids to weather conditions a few weeks prior to harvest largely depended on genetic factors. It can be concluded that the environmental response of pungency-related compounds is strongly influenced by the interaction of two major abiotic factors: air temperature and precipitation. Air temperature has been shown to influence the expression of genes responsible for capsaicin biosynthesis in different pepper cultivars. González-Zamora et al. (2013) reported that increased air temperature elevates capsaicin and dihydrocapsaicin levels. Yang et al. (2024), reviewing multiple studies, concluded that high temperatures increased capsaicinoid content in many, but not all, Capsicum genotypes. In this study, the high capsaicin and dihydrocapsaicin concentrations measured in 2022, when radiation intensity and maximum temperatures exceeded 40 °C a few days prior to the first harvest, support these observations.

Another climatic factor contributing to low capsaicinoid levels in 2023 could be high precipitation. It has been shown that excessive irrigation or heavy rainfall can reduce capsaicinoid content in certain genotypes. One analytical parameter useful for understanding capsaicinoid variation in terms of genetic variation and climatic changes is the capsaicin/dihydrocapsaicin ratio.

The weather differences between the first and second harvests in 2022 and 2023 did not result in significant changes in this ratio for any genotype studied.

Variation in the capsaicin/dihydrocapsaicin ratio was more closely associated with pre-harvest precipitation than with air temperature. The results support the observation that high water availability reduces the activity of enzymes regulating capsaicinoid biosynthesis in some hot pepper cultivars. The drastic reduction in precipitation before the second harvest of 2023 resulted in a significant increase in this ratio compared to the first harvest, confirming that mild drought (low water availability) favorably affects the activity of capsaicin-synthesizing enzymes (capsaicin synthase) while partially reducing dihydrocapsaicin-synthase activity (Ruiz-Lau et al., 2011; Yang et al., 2024).

The results confirm that in highly pungent peppers, C-vitamin biosynthesis is significantly influenced by increased water availability due to precipitation or irrigation, whereas in low- to medium-pungency cultivars, reduced water supply or mild drought promotes C-vitamin accumulation. The positive correlation between water availability and C-vitamin content does not align with the earlier findings of Duah et al. (2021b), which suggested an inverse relationship between water supply and C-vitamin levels. In fact, increased precipitation leads to higher soil moisture, which has previously been associated with greater C-vitamin accumulation in chili peppers. In 2022, exposure to high sunlight intensity, during which air temperatures exceeded 40 °C for several consecutive days, may have contributed to the enhancement of biochemical factors regulating C-vitamin synthesis.

5. New scientific findings

- The content of fat-soluble phytochemicals in hot peppers was primarily influenced by seasonal variations in weather conditions. Most yellow xanthophylls were esterified as monoesters. Differences between cultivars can be attributed to pre-harvest weather effects, which affected the esterification process.
- Genotype had a major effect on carotenoid and tocopherol content.
- The results indicated that the degree of E-vitamin oxidation mainly depends on genetic factors controlling the oxidation-reduction pathways.
- Changes in capsaicinoid and vitamin C content, as well as their responses to meteorological factors, were significantly influenced not only by genotype but also by the interaction between genotype and year effect.
- The sensitivity of phytochemical components in hot peppers to year effects is genotype-dependent; the different precipitation levels measured during the 2022 and 2023 growing seasons (154 mm and 437 mm, respectively) and the average pre-harvest temperature (significantly higher in 2023, $p < 0.05$) could induce either favorable or unfavorable changes.

6. Publications related to the thesis topic

Journal articles with impact factor

I.M. Gyalai, L. Helyes, H.G. Daood, F. Kovács, A. Szarvas, F. Lantos (2025). Genetic and seasonal factors influence pungent pepper capsaicinoid and vitamin C content. Horticulturae 11 (3), 286. (Q1) https://doi.org/10.3390/horticulturae11030286

I.M. Gyalai, F. Lantos, H.G. Daood, F. Kovács, A. Szarvas, L. Helyes (2025). Carotenoid and tocopherol content of pungent spice red peppers as affected by genetic and abiotic factors. Food Chemistry Advances 7 (3), 100987. (Q1) https://doi.org/10.1016/j.focha.2025.100987

Other scientific articles

Lantos F., Makra L., Mike K., Gyalai I.M. (2022). SPAD values, as well as sugar- and capsaicin content indifferent varieties of outdoor peppers. COLUMELLA: JOURNAL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, 9 (1). pp. 5-15. ISSN 2064-7816 (2022).

Conference proceedings

Gyalai I.M., Lantos F. (2021). Effect of nutrient solution with different N:K ratios on the yield of Claudius F1 sprouted peppers in container cultivation. RESEARCH JOURNAL OF AGRICULTURAL SCIENCE, 53 (4). pp. 75-82. ISSN 2066-1843 (2021).

Gyalai I.M. (2021). A kapszaicin antioxidáns hatásának vizsgálatai. Mezőgazdasági és vidékfejlesztési kutatások a jövő szolgálatában 2. Magyar Tudományos Akadémia Szegedi Akadémiai Bizottság Mezőgazdasági Szakbizottság, p. 10. (2021).

Gyalai I.M. (2023). Különböző csípős paprika fajták beltartalmi elemeinek vizsgálata. Magyar Tudományos Akadémia Szegedi Akadémiai Bizottság, Mezőgazdasági Szakbizottság.