

Hungarian University of Agriculture and Life Sciences Doctoral School for Plant Sciences

Impact of Water Supply, Harvest and Drying Methods on Phytochemical Content of New Chili Pepper Hybrids

Thesis of PhD Dissertation

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1. BACKGROUND AND OBJECTIVES

1.1 Importance and background

In the Solanaceae family, the genus Capsicum contains more than 30 species of chili peppers, which are non-climacteric fruits. Chili peppers originated in the tropical and subtropical America, Central and South America regions, with tropical and warm climate. At the end of the sixteenth century, the chili had arrived and started to spread by Ginnie [Guinea] to India, and Europe mainly to Spain and Italy. Chili began to spread quickly in the south and east of European territories (Bosland et al. 2012).

Only five species have been domesticated and became common over the years; they are *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens* (Pickersgill, 1997). Based on its aromatic, coloring, and flavoring qualities, *C. annum* is the species with the highest commercial demand in the food industry (Vega-Galvez et al. 2008; Arslan and Özcan 2011).

World production of fresh and dry peppers is about 36 and 4.8 million tons per year, in a total area of 2.06 and 1.62 million hectares, respectively (FAO 2021). One of the 200 primary commodities are chili peppers (Food and Agriculture Organization of the United Nations, 2007). China, Vietnam, and India produce the most chili peppers worldwide. Brazil and Indonesia together produce more than 80% of the world's goods (Farias et al. 2020).

In Hungary's towns of Kalocsa and Szeged, chili pepper production became a major industry because of favorable weather, a hot environment, and fertile soil (Bosland et al. 2012). The harvested area, or the region from which a crop is taken, and global production both increased over the past five years.

Even though the area harvest in Eastern Europe has been decreasing since 2017 (113062 ha) and in 2018 (100422 ha) and 2019 (103224 ha), production has

been increased in 2017 (3275545 tones), 2018 (3215936 tones) and 2019 (3439339 tones).

The FAO reports affirmed that since 2017 the area harvest and output in Hungary have been declining. The production of chilies was 98880 tons in 2018 and 2019 (91160 tones). Hungary, along with Serbia, Croatia, Spain, and Macedonia, are among the five nations in Europe with the biggest production of chili peppers, despite that fact, the area harvest has been declining. Depending on the pepper cultivar developed, chili paprika can be non-pungent, sweet, and pungent, or scorching (Vinkonic et al. 2018).

Chili peppers are the most common vegetable and spice crop consumed today and go by many different names, including pepper, chili, chile, chilli, chili pepper, aji, rocoto, paprika, and capsicum (Kothari et al. 2010). They are also available in a variety of forms, including fresh, dried, and processed goods.

The chili pepper is renowned in the culinary world for its color, flavor, texture, and scent (fragrance). As a result, it is employed in the food processing sector to create a variety of goods, including sausage, beef, cheese, butter, salad, condiment blends, desserts, and processed meals (Govindarajan, 1985). Due to the bioactive components found in chili peppers, they are frequently used in a variety of foods, including dairy products, salads, different salsas, baked goods, sweets, cosmetics, and medications. (Bosland et al., 2012).

The interest in bioactive compounds from raw materials and extracts as natural ingredients for cosmetic and pharmaceutical applications has increased due to consumer and industry behavior that is ecologically responsible. This new development also produces products that have fewer allergic reactions and are more advantageous to users (Baenas et al. 2019).

Instead of using synthetic compounds the industry has been looking for natural ingredients, so the pharmaceutical and cosmetic industry has been using the main compounds in chili for cosmetic product as shampoo, soaps, make-up, gel, cream, lotion for pains disorders, neuropathy, headache, trigeminal neuralgia and herpes zoster and medicine to prevent cold, sinus infection, sore throat and improve digestion and blood circulation (Khare, 2004).

1.2 Objectives

The main objective of this study was to evaluate the production of four different chili pepper cultivars in two consecutive seasons with special focus on the quality components and phytonutrients presents in the fruits of out-door cultivated chili peppers under the influence of abiotic factors (temperature, rainfall, humidity), and to study the response of such cultivars to different drying methods.

- To analyze, by developed chromatographic methods, the composition and content of phytochemicals (vitamin C, capsaicinoids, carotenoids and tocopherols) present in new cultivars of chili pepper.
- 2. To Investigate how the phytochemicals respond to the changes in the abiotic factors during out-door cultivation.in two consecutive years.
- 3. To study the response of the phytochemicals to natural and thermal drying methods with respect to stability of vitamin C, capsaicinoids, carotenoids and tocopherols in three different chili pepper cultivars.

2. MATERIALS AND METHODS

The influence of physiological variables and phytochemical responses of chili pepper cultivars under three distinct water supply (WS) treatments were examined over the production phase of the experiment. The research was carried out over two years (2019-2020) in open field growing at the Horticulture Institute experimental field, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary (latitude 47°61' N, long. 19°32' E).

WS treatment was 0% or control (considering natural precipitation), 50% deficit irrigation and 100% optimal drip WS. In the drying experiment, the pepper

was dried for 2.5 hours at 90 °C, followed by 2.5 hours at 70 °C, 2.5 hours at 50 °C, and lastly 2.5 hours at air temperature. Irrigation was set up using a drip system for both experimental seasons. A pressure gauge and water meter were installed with control valves in each treatment to manually adjust the water pressure, depth of WS, and uniformity of water and distribution. The crop water requirement (ETc) was measured based on the AquaCrop model by Food and Agriculture Organization to determine evapotranspiration (ETo) using the Penman-Monteith method corrected by a crop coefficient (Kc) (Takács et al., 2019). The chili cultivars were given three different WS treatments; control (0%) except for natural precipitation with no regular irrigation, deficit irrigation (50%), and optimum WS (100%). The experimental design used was randomized blocks (RCBD) with four replications for each WS treatment. The pepper cultivars were Hetényi Parázs (HET), Unikal (UNIK), Unijol (UNIJ) and Habanero (HAB).

Between the two years of the experiment, there was a substantial difference: in 2019, the mean temperature was 20.3 °C, and there was 132.6 mm of precipitation. In contrast, in 2020, the mean temperature was 18.9 °C, and there was 478.6 mm of precipitation. According to the climatic conditions and state of the fruits, there were 4 harvest times in 2019 and 3 harvests in 2020. The total production of fruits per plant was obtained by manually harvest between August and October in both years. The factors measured were relative chlorophyll content (expressed as SPAD values), chlorophyll fluorescence (Fv/Fm), canopy temperature and soil water content (v/v%).

Analyzes performed on high performance liquid chromatography (HPLC) equipment according to each protocol. All analytical grade solvents and chemicals, as well as High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) grade organic solvents used in the analyses. Standard capsaicin 95% (CAP), nor-dihydrocapsaicin 95% (NDC) and dihydrocapsaicin 85% (DC), zeaxanthin 95%, β -carotene 93%, 8- β -apo-carotenal 96%, D- α -

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tocopherol 95.5% (α -T), γ -tocopherol 96% (γ -T), D- α -tocopherol acetate 96% (α -TES), and β -tocopherol 50 mg/ml (β -T) were from Sigma- Aldrich via Merck (Budapest, Hungary). The α -tocopherol quinone (α -TQ) and its reduced form (α -TQH2) were prepared from standard α -T by oxidation with FeCl3 followed by reduction with NaBH4 in ethanol according to Kruk et al. (2008).

The HPLC separation of capsaicinoids was performed on a Cross-Linked Nucleodur C18, 150 x 4.6 mm, 3um column (ISIS, from Machery Nagel, Dürer, Germany) with an isocratic elution of 50:50 water: acetonitrile and a flow rate of 0.8 mL/min. The compounds were detected fluorometrically at EX: 280 nm and EM: 320 nm. Peaks corresponding to the different capsaicinoid compounds were identified based on their retention time and mass data from LC-MS/MS analysis as compared to standard materials analysed by the same method (Daood et al. 2015).

Vitamin C content was determined according to the method and HPLC protocols of Nagy et al. (2015). L-ascorbic acid (vitamin C) was separated from other organic acids on aqua C18, 3μ , 150 x 4,6 mm column (Nautilus from Machery Nagel, Dürer, Germany) using a gradient elution of acetonitrile in 0.01M KH2PO4 with a flow rate of 0.7 mL/min and DAD detection between 190 and 400 nm. For quantification of vitamin C, the peak area was integrated at 244 nm. Identification of vitamin C was based on the comparison of retention time and spectral characteristics with those of standard solution. Quantification was based on calibration of vitamin C concentration and integrated peak area. The calibration curve was drawn between 0 and 120 µg/mL.

Separation of carotenoids was performed on Nucleosil C-18, 3μ , 240x4.6 mm column (Macherey-Nagel GmbH, Dueren, Germany) with gradient elution consisting of (A): Water, (B) methanol and (C) 10:55:35 methanol-isopropanol-acetonitrile. The elution started with 8%, A in B, changed to 100% B in 3 minutes and then to 100% C in 30 minutes, which stayed isocratic for 5 minutes and turned

to 8% A in B% A in 5. The flow rate was 0.6 ml/min, and carotenoids were detected between 190 and 700 nm using a diode-array detector. To achieve simultaneous determination of both carotenoids and tocopherols the fluorescent detector was also operated with the DAD. The detection of tocopherol was at 295nm Excitation and 325 nm Emission. Identification of all carotenoid compounds in the pepper cultivars was made using the liquid chromatography-diode array detection-mass spectrometry (LC-DAD-MS) as described previously by Duah et al. (2021).

In heat drying treatments, 3 kg of pods from each cultivar were taken in triplicate and sliced apart (split in half) using a stainless-steel knife to aid dehydration, as is done in industrial drying. In the case of natural drying, the complete pods were dried without shredding or mincing, as is common in small-scale drying around farms. Using a programmable drying chamber with air circulation, two ways were used to thermally dehydrate the pods. The pods were dried at 60 °C for 30 hours in one way (Thermal 60 °C). A stepwise thermal program was used in the other technique, commencing with 90 °C for 2.5 hours, then 70 °C for 2.5 hours, 50 °C for 2.5 hours, and finally air for 2.5 hours.

Data were expressed as the mean \pm standard deviation (SD) among physiological responses, pepper cultivars, WS treatments, and phytonutrients. The Kolmogorov-Smirnov test was used to decide if samples come from populations with a normal distribution. Levene's test was used to test the variance's homoscedasticity, where the null hypothesis is that the variances within each of the examined groups are the same. One-way analysis of variance (ANOVA) was used to examine the effect of WS.

3. RESULTS AND DISCUSSIONS

Based on the statistical analysis, the results were rigorously analyzed and described in the results and discussion section. Different results were obtained in

the two years of analysis 2019 and 2020 according to the analyzed variety HET, UNIK, UNIJ and HAB. In general, and in the two years of studies, the chili pepper least adapted to the climatic conditions of the study was the Habanero (*C. chinense*), which responded poorly to irrigation and to climatic conditions in general.

3.1 Yield

The UNIJ and UNIK cultures showed similar behaviour for some compounds during the study period. In general, the marketable yield in 2019 was the highest for HET 0% (24.67 \pm 3.34 t/ha) and the lowest for HAB 50% (3.36 \pm 0.47 t/ha), while in 2020 the highest production was for UNIJ 100% (33.58 \pm 1.55 t/ha) and the lowest for HAB 0% (9.03 \pm 3.09 t/ha). Overall, UNIJ had the highest production and HAB the lowest output in 2020. The highest total yield was in the cultivar UNIJ with annual mean of 27,50 (t/ha). The cultivar HAB had the lowest yield at the 1st harvest, this is most probably due to that the weather conditions for the flowering of habanero plants were late. Having become suitable conditions for flowering the yield of HAB was better at the 2nd harvest (6.4 2 t/ha) and then dropped to 0.53 (t/ha) at the third harvest as a response to the cool weather.

3.2 Capsaicinoids

Regarding secondary phytonutrient compounds in fresh chili pepper, there was a variation between the two years. Due to high rainfall in 2020 the treatment WS 0%, 50% and 100% did not have significant effects due to abundant water in the soil even for the control treatment. In 2019, we had more significant results when it came to WS treatment effects. In 2019 concentration of the major capsaicinoids was highest for the UNIJ variety peaking at 2743.1±429 μ g/g, 2920.7±567.1 μ g/g and 269.5±37 μ g/g respectively for capsaicin, dihydrocapsaicin and nordihydrocapsaicin. In 2020 UNIJ also showed the highest concentrations for

capsaicin 2875.77 ±411.00 μ g/g, dihydrocapsaicin 1341.31±135.23 μ g/g and nordihydrocapsaicin. 176.14±12.93 μ g/g.

3.3 Carotenoids

The total carotenoid content was the highest for HET at all four harvests in 2019 and the lowest for HAB. In 2020 when the rainfall was higher, the biggest difference was found in the amount of total carotenoids among the varieties during the harvest time. In 2019 vitamin C had higher concentrations for all varieties at the fourth harvest, with the highest concentrations occurring at 0% WS, for HET (4397.5±76.9 µg/g), for UNIK (4184.0±286.2 µg/g), for UNIJ (3842.3±96.7 µg/g) and for HAB (3223.7±118.6 µg/g). In 2020, there were no significant differences between the harvest times and between the WS treatments. The maximum and the minimum vitamin C values occurred in UNIK 0% (4855.63 ± 365.56 µg/g) and UNIJ 100% (1369.88 ± 198.90 µg/g), respectively.

3.4 Tocopherols

Based on our findings, in 2019 α -tocopherol the maximum and the minimum α tocopherol concentrations were the followings. For HET the highest concentration was 69.0±1.71 in 50% and the lowest in 0% 13.3±12.74 µg/g, for UNIK the highest and lowest concentration were respectively 15.4±4.00 µg/g in 0% and 60.0±3.39 µg/g in 50%. For UNIK there was a minimum of 1.4±2.01 µg/g 0% and a maximum of 50.4±5.0 µg/g 0% at the 2nd harvest. As for HAB there the maximum was 1.7±0.78 µg/g in 50% and the minimum 0.1±0.03 µg/g for all WS treatments at the 1st harvest. In 2020, the concentration of α -Toc was higher compared to the values of 2019, with the maximum and minimum being 81.39 ± 6.40 µg/g in UNIK 50% and for HAB 50% (0.16 ± 0.02 µg/g), respectively.

3.5 Impact of drying methods

The capsaicinoid concentration of the examined hybrids' raw materials varied greatly, ranging from the lowest (3.540.28 71mg.g⁻¹dwt) in UNIK to the highest (31.472.71 mg. g¹dwt) in Unijol.

For dry samples, UNIJ, which had the highest amount of capsaicinoids, had the best stability, with capsaicinoid retention ranging from 67 to 93 percent, while UNIK, which had the lowest level of capsaicinoids, had the lowest stability, with retention ranging from 35 to 46 percent. The high antioxidant content (primarily vitamin C and flavonoids) in such genotypes has been related to the considerably increased stability of capsaicinoids in highly spicy peppers (Maurya et al. 2018). Naturally dried peppers contained the highest levels of pungent compounds than thermally dried peppers, according to (Bianchi, G. and Scalzo, R. (2018); Topuz, A. et al. (2011). The total capsaicinoid concentration of naturally dried HET and UNIJ was not significantly different from the amounts measured in the raw materials. The resilience of capsaicinoids under thermal drying conditions differed among cultivars, with UNIJ and UNIK losing their pungency regardless of temperature or time applied.

Thermal drying or dehydration is the most critical step of pepper processing since carotenoids are easily degraded by heat particularly in presence of molecular oxygen. Therefore, such a step should be optimized to allow the minimal deterioration to the carotenoid pigments, which are responsible for the attractive color of the seasoning and chili pepper products. Also, UNIK saw the greatest color loss after both natural and thermal drying, despite HET having the highest original carotenoid content. Pigment retention was 43-53% for HET and 52-77% for UNIK when compared to the content in the raw material before drying. Yellow-colored xanthophylls responded to natural drying similarly to red pigments, especially in terms of free, mono-, and diester levels. In general, I could

conclude that for carotenoids the high retention necessitated the use of low temperature drying, regardless of the drying time.

The initial level of tocopherols before drying differed considerably (P < 0.05) amongst genotypes, with UNIK being the richest. The raw components of UNIJ, the hybrid with the highest degrees of pungency, had the lowest concentration. Surprisingly, there was an inverse link between carotenoid content and pungency based on drying stability. The inverse association identified with different chilis having varied colors and capsaicinoids could be due to the synergic or antagonist relationship between distinct metabolites (Kim et al. 2017). The percentage of total tocopherols retained was found to be greater in UNIJ, followed by HET, and finally UNIK. Tocopherol retention was 83-92% for UNIJI, 72-79% for HET, and 63-73% for UNIK. Bianchi and Scalzo (2018) reported 61.5%, 63.4%, and 48.3% for chili peppers dried at 50, 57, and 64°C, respectively. Tocopherol stability in spice red peppers have been reported to be influenced by genotype, ripeness state before drying, and drying method in earlier investigations (Howard and Wildman, 2007). The response of the various tocopherol compounds to drying treatments is shown in Table 16. Most tocopherol components changed in a similar way to the total, with some variance in the inter-conversion between unoxidized and oxidized molecules, such as the conversion of α-Toc-Es toα -Toc HQ-Es. Except for α -Toc HQ-Es, the highest concentrations of tocopherol compounds were found in the raw materials before drying and were reduced by natural and heat drying. The reduced form of oxidized-tocopherol ester was present in extremely low concentrations in raw materials of all cultivars but increased considerably upon drying. Because of the stabilizing impact of the ester moiety on the inverse reaction of α -Toc HQ-Es, it is substantially more resistant to thermal breakdown than α -TocHQ. This study backs with findings of Kruk et al. (2014) about high antioxidant reactivity of tocopherol hydroquinone.

4. CONCLUSIONS AND RECOMMENDATIONS

> The impact of harvest depends extremely on the external climate factors such as air temperature, precipitation, and sunshine period, and on the saturation of water or the drought water stress in the soil, thereby affecting the metabolism and the production of secondary compost of the chili plants.

- The level of rainfall in 2020 was much higher (three times more) and the temperature was lower than in 2019, moreover, therefore there was a marked decline in the canopy temperature for all crop production. The canopy temperature has one negative correlation with the soil moisture, and this could be more visible in 2019 with lower rainfall during crop production.
- Based on our findings temporal factors were decisive for maturation and fruit collection, between the four genotypes the habanero starts flowering later than the others in 2019, and in 2020 because of the rainy weather, the chili flowering and harvest were late. This means how that weather conditions such as temperature; precipitation is important for the first months after planting. The habanero was the genotype chili that was more affected by the environmental factors, in both years the Habanero have less value for SPAD.
- The WS when increased to 50% and100% caused the content of the major and some minor capsaicinoids to significantly decrease particularly at the 2nd, 3rd or the last harvest, while no change or an increase was observed at the 1st harvest with the increase in WS. Since the main difference between the harvest periods is in the climate variable like temperature, precipitation and sunshine, the response of capsaicinoids to harvest time is most probably climatedependent rather than genotype-dependent.
- Biosynthesis of capsaicinoids in the new cultivars of chili pepper may favor the conditions of the late harvest when the temperature throughout the day is low with minimal precipitation. This concept is supported by the fact that at the late harvests the peppers from different genotypes contained significantly

higher amounts of the pungent materials than the other harvests particularly with no WS (0%) applied. If the main goal of the research is to obtain higher amounts pungent materials from chili peppers, it is recommended to perform late harvest at the end of the cultivation season.

- > The UNIJ, is the variety that has the highest stability of carotenoid and capsaicinoids. while, the UNIK had the lowest level of capsaicinoids, and had the lowest stability with retention ranging from 35 to 45 percent. For total tocopherols retained were found to be greater in UNIJ, followed by HET, and finally UNIK. According to this, the UNIJ, is the variety that is more recommended for the drying process in the chili industry.
- \triangleright Although the new hybrids lost significant amount of their phytochemicals during drying, they contained high concentrations of such bioactive compounds making them great ingredients for the manufacture of products of exceptional quality and nutritional worth. Natural drying has been shown to result in the least loss of all phytochemicals, however, it is recommended to utilize a hightemperature-short-time drying technique to create safe spices by preventing mold growth and toxification during storage. It's also worth noting that the loss of bioactive components in UNIK and UNIJ cultivars after heat drying at 60°C or 90-25°C is acceptable in the mass manufacture of dry spice chili pepper. To manufacture safe spice chilis with remarkable color and flavor, a mixture of thermally dried UNIK and UNIJ products is recommended. Because pretreatments prior to drying are difficult to implement and cost-effective in large-scale manufacturing, additional quality enhancement of spicy chili products should be achieved by optimizing drying conditions (temperature and time).

5. NEW SCIENTIFIC RESULTS

- With the application of HPLC-MS/MS technique for the detection and identification of carotenoid fatty acid esters, it could be confirmed that some diesters of yellow and red xanthophylls in chili peppers examined contain unsaturated fatty acids moieties. Such unsaturated fatty acids may cause the storage stability of chili carotenoids, if not well controlled, to decrease at postharvest processing and storage of chili peppers.
- 2) For the first time the degradation product of vitamin E in the native (non-saponified) extract of chili peppers could be identified as α-tocopherol hydro-quinone, not quinone, indicating the high hydrogen donning capacity of chili pepper. The ratio of α-tocopherol/ α-tocopherol hydroquinone can be used as an index for estimating the state of reduction-oxidation potential in many crops, including spice peppers and chili.
- 3) The correlation between the leaf temperature and the soil moisture was studied for the first time for chili peppers under the cultivation conditions. For all cultivar examined, although a weak correlation with R²=0.4289 was found the optimum soil moisture to prevent the detrimental raise in leaf temperature could be estimated to be around 20 v/v%.
- 4) It was found that the water stress significantly increased the yield in the 1st harvest for all cultivar 'Hetényi Parázs', 'Unikal', 'Unijol', 'Habanero' particularly in 2020 when the precipitation was substantially higher.
- 5) It was affirmed that the high-WS particularly with the accumulated precipitation influences positively the capsaicinoid concentration, but it was not favorable for the biosynthesis of other components like vitamin C, vitamin E and carotenoids. This held true in most cultivar studied except HAB, in which the highest WS promoted to a high extent, the biosynthesis of carotenoids.

- 6) The relatively cool weather at the last harvest caused the carotenoid content in the less pungent cultivars to significantly decrease, and an increase in the highly pungent ones it increased the amounts of carotenoids, particularly the yellow-colored pigments including the provitamin A, compounds especially βcarotene.
- 7) The cool climate of the last harvest was found favorable for the synthesis of the major capsaicinoids and more interestingly of vitamin C in the new hybrid Unijol, On the other hand, the impact of increased WS (Stress) on phytonutrients were found to variable according to the interaction with harvesting time and genotypes.
- 8) There was a significant difference between the different cultivars in their response to thermal and natural drying, with the highly pungent Unijol being of the highest stability. It was also affirmed that high levels of capsaicinoids in chili peppers may stands beyond the reason for high stability of carotenoids, and antioxidants during thermal drying of spice chili peppers.

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6. PUBLICATIONS RELATED TO THE TOPIC OF THE THESIS

IF, SCI journal papers

- <u>Silva e Souza, C.</u>; Daood, H.; Agyemang Duah, S.; Vinogradov, S.; Palotás, G.; Neményi, A.; Helyes, L.; Pék, Z. Stability of carotenoids, carotenoid esters, tocopherols and capsaicinoids in new chili pepper hybrids during natural and thermal drying. *LWT Food Sci. Technol.* 2022, *163*, 113520, doi:10.1016/j.lwt.2022.113520.
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Peer-reviewed articles in Hungarian that are not referenced or registered

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