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

Clarice Silva e Souza

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The Doctoral School Title: Doctoral School of Plant Sciences

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Head: Professor Dr. Lajos Helyes
Head of Doctoral schools of the Hungarian University of Agricultural and Life Sciences
Director of Institute of Horticultural Sciences
Hungarian University of Agriculture and Life Sciences

Supervisors: Professor Dr. Zoltán Pek
Institute of Horticultural Sciences
Hungarian University of Agriculture and Life Sciences

Professor Dr. Hussein G. Daood
Institute of Horticultural Sciences, Analytical Laboratories
Hungarian University of Agriculture and Life Sciences

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Approval of the Head of Doctoral School

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Approval of the Supervisor **Approval of the Supervisor**

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List of legend and abbreviations

CAP: Capsaicin

Caps: capsanthin

Capsr: Capsorubin

DC: Dihydrocapsaicin

DE: di-ester

ESI: Electro Spray Ionization

EU: European Union

HAB: Habanero

HCAP: Homocapsaicin

HDCs Homodihydrocapsaicins

HET: 'Hetényi Parázs'

HPLC: High-Performance Liquid Chromatography

IAEA: International Atomic Energy Agency

iDC: dihydrocapsaicin isomer

LC-DAD-MS: Liquid Chromatography-Diode Array Detection-Mass Spectrometry

ME: mono-ester

NDC: Nordihydrocapsaicin

PSII: Photosystem II

PTFE: Poly Tetrafluoro Ethylene

R: Red

SHU: Scoville heat units

SPAD: Soil Plant Analysis Development

T: Total

Toc: Tocopherol

TocHQ: Tocopherol hydroquinone

TocQ: Tocopherol quinine

UNIJ: Unijol

UNIK: Unikal

WST: Water supply treatment

X: Xanthophyll

Y: Yellow

Zeax: Zeaxanthin

1. INTRODUCTION

Chili pepper (*Capsicum*. sp) from Solanaceae family, gender capsicum originated in the tropical and subtropical America, Central and South region have more than 30 species. and have been used around the world. The chili pepper is well-known in the culinary world for its color, flavor, texture, and scent (fragrance). Recently, it is widely used in the food processing sector to create a variety of goods, including processed foods, sausage, meat, cheese, butter, salad, and condiment combinations (Govindarajan, 1985). Due to the considerably high amounts of bioactive components in the fruits of chili peppers, they are frequently used as ingredients in a variety of foods, including dairy products, salads, different salsas, baked goods, candies, cosmetics, and medications. (Boland et al. 2012). Due to it high content of pungent materials, capsaicinoids, chili peppers received great interest and attention. The nutritional and therapeutical properties of the chili peppers caused the farmers to increase it production all over the word, thereby playing significant role in the development of agricultural economy.

In the last decades, there were new trends to improve the quality features of chili peppers by biotechnological methods including breeding to create disease-resistant varieties and hybrids that also suit the requirements of the food and chemical industries. The environmental and agricultural condition are to be optimized considering the necessities of the traditional or new chili peppers for each industry. In the processing of some products of chili pepper, the crop should be overripen and then dried to produce powders with high quality. Drying is the most critical processing step, which should be performed adequately to minimize the loss of quality and biological activity of the chili pepper powder. Depending on weather conditions such temperature, air moisture and sunshine, the chili pepper fruits can be naturally or thermally dried with or without combination of other tools such as microwave and ultrasound prior to dehydration.

Because of global warming and substantial variation in the climate from year to year, it has become essentially needed to study the impact of different stresses on the quality attributes and the level of bioactive compounds in the chili pepper fruits. Among stresses that threat agricultural crops including chili peppers salt, drought, and water stress. The effect of such stresses on the quality and bioactivity of any crop can be sustained or depressed by the changes in the other environmental factors like storms, warmness, period of sunshine and precipitation.

The problems encountered in the production and cultivation of the different chili peppers my include:

1. Real analytical data on the composition and content of the quality and nutritional attributes in the different chili peppers are missing.
2. It is not clarified yet that whether fruits of each chili pepper cultivar need to be overripen before processing and what are the conditions under which the overripening to be performed.
3. The response of the different new cultivars to the water stress with respect to quality and nutritional attributes is not well studied
4. No information is available on stability of the different cultivars during natural and thermal drying at different temperatures.

General 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.

Specific objectives

1. 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. LITERATURE OVERVIEW

2.1. Economic importance

Chili peppers, which are non-climacteric fruit from the family Solanaceae and the gender is Capsicum with more than 30 species. 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 are common over the years; they are *C. annuum, C. chinense, C. frutescens, C. baccatum, and C. pubescens* (Pickersgill, B. 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; Aslan and Ozcan 2011).
World production of fresh and dry peppers is about 36 and 4.6 million tons per year, in a total area of 1.9 and 1.85 million hectares, respectively (FAO 2017).

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 area harvest, 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.

Due to its color, flavor, texture, and aroma (fragrance), the chili pepper is well-known in the culinary world. As a result, it is used in the food processing industry to make a variety of products, including sausage, meat, cheese, butter, salad, condiment mixtures, deserts, and processed foods (Govindarajan, 1985). The chili pepper is a common ingredient in many dishes, including dairy products, salads, various salsas and salads, baked goods, candies, cosmetics, and pharmaceuticals because of the bioactive compounds present in the chili peppers. (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 use synthetic compounds the industry has been looking for natural ingredients, so the pharmaceutical and cosmetic industry has been using the mains 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).

2.2. Biological importance

The name of gender Capsicum comes from the major representatives in the group capsaicin and dihydrocapsaicin (Aza-González, C. et al. 2011). In Greek work “Kapismo” mean to bite (Basu and De, 2003). Capsicum is one alkaloid compound responsible for the hot and pungent taste (Hayman M and Kam, 2008; Baenas et al. 2019), and responsible for the irritant in the mammalian skin (Frias a

nd Merighi. 2006). Being spicy has a clear evolutionary advantage for plants because capsaicinoids have extensive antibacterial and antifungal properties that help preserve the vital seeds from contamination (Bhatla and Lal, 2018).

The chili not only enhance the test, aroma, flavors and color of the food or the culinary, the vegetable is a rich source of bioactive components such as capsaicin, vitamins C and E and provitamin A,

carotenoids, flavonoids (quercetin, luteolin and phenolic acids) (Palma, J.M. et al. 2015), and minerals, including iron, calcium and manganese that can be contributing for the human diet (Baenas et al. 2019). The beneficial effects of the chili have long been documented.

Clinical investigations of topical capsaicin include assessment in chronic pain syndromes viz. rheumatoid arthritis, postherpetic neuralgia, hemodialysis-associated itching, reflex sympathetic dystrophy syndrome, diabetic neuropathy, psoriasis, vulvar vestibulitis and post mastectomy neuroma (Rumsfield and West, 1991).

Due to its secondary chemicals and inherent insecticidal, anthelmintic, and larvicidal properties, chili peppers have become more and more popular as agroecology has advanced. Rhyzopertha Dominica and Sitophilus granaries are two insects that have been successfully treated with the chili pepper extract *C. frutescens* (Oni M. 2011).

2.3. Phytochemicals in chili peppers

The bioactive compounds present in natural matrices have received an increased interest in the last decades. Under this perspective, the research have focused on the matrices that can provide extracts for the technology to isolate and highly purify the compounds, which are subsequently integrated for industrial use in food pharma formulations and products (Baenas et al. 2019). According to Jayaprakasha, et al. 2012 the species, the fruit part (placenta, pericarp, and seeds), the cultivar, ripening stage, the climatic, storage conditions and processing practices are factors that can interfere in the content of bioactive compounds.

Generally, fruits and vegetables are great providers of vitamins (A, C, E, D, and B), carotenoids, capsaicinoids, phenolics, and minerals that are part of our daily diet. Due to their bioactive properties, some spices, including turmeric, black cumin, ginger, garlic, saffron, black pepper, and chili pepper, have been utilized for both prevention and treatment (Zheng et al. 2016). Chili peppers are a wonderful source of vitamins and secondary compounds. They are also a good choice to supplement our regular diets because of their many health advantages.

2.3.1. Capsaicindois

The primary components of flavor of chili peppers are capsaicinoids, which are alkaloids and determine the pungency of the pepper by way of a benzene ring and being modified by an acyl chain

(Xiang et al. 2021; Ye et al. 2020). Capsaicin and 6,7-dihydrocapsaicin make up the majority of the capsaicinoids, with nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin being the least abundant (Jackson 2007).

Capsaicin and dihydrocapsaicin are responsible for 90% of the spice, that compounds give the mammalian the heat sensation in the mucosa and the mouth (Huang et al. 2014). Recent studies have discovered capsaicidoids compounds with non-pungent, which are capsinoids (capsiate, dihydrocapsiate, and nordihydrocapsiate) and capsiconinoids (capsiconiate and dihydrocapsiconiate) (Gupta et al. 2021).

The pungent capsaicin and non-pungent capsiate are two groups of compounds having different structures and biosynthetic precursors from capsaicin, and the individual of pungent capsaicinoids are charactered in the molecule with a nitrogen atom (amide group) according to Gupta et al. 2021.

Capsaicin provides anti-inflammatory, anticancer, and antioxidant properties that are beneficial to health. The advantages for people relate to gastrointestinal symptoms, cancer (prostate, stomach, breast, and pancreatic cancer), dermatological problems (Patowary et al. 2017), obesity, renal failure, neuropathic pain, vulva vestibulitis syndrome, and urinary system disorders (Xiang et al. 2021).

The number of capsaicin and capsiates in fresh chili peppers at various stages of maturation varies while the fruit ripens under the same controlled conditions. The amount of capsaicin increases from the first days of maturation until a maximum value, and then the trend reverses and the capsaicin content decrease after that day (Mercedes et al. 2020; Barbero et al. 2016). After the fruits have produced their maximum amount of capsaicin, they begin to diminish, and this phenomenon is linked to the enzyme peroxidase, which begins to oxidize the compounds dihydrocapsaicin and capsaicin (Bernal et al. 1993).

Red chili peppers have more capsaicin than green ones because they are spicier, and the quantity of the chemicals might vary depending on the kind.(Ye et al. 2022). The capsaicin start is synthetized when the fruit is green and during the maturation the capsaicin begins to accumulate and has the maximum level when it turns red (Ananthan et al. 2018; Barbero et al. 2014). The tissues that house the seeds, called the pericarp, contain the highest concentrations of capsaicin, while the seeds and peduncle contain the lowest concentrations (Buczkowska et al. 2016).

Studies have been conducted for know how the factors affect capsaicin compounds and found the best combination for each purpose. Nagy et al. (2018) studied the extraction efficiency of the capsaicin compounds with different solvent. The authors applied reversed-phase HPLC method to

choose the best technique for extraction of different capsaicinoids. Other researchers (Antonious et al. 2009) examined the origins of capsaicinoids in chili peppers in eight different nations and discovered that accessions from Mexico, the United States, and Brazil had the highest levels of total capsaicinoids.

2.3.2. Carotenoids

In the chemistry vision, the carotenoids are based on a C40 terpenoid skeleton are lipophilic compounds, soluble in hydrophobic substances, organic solvent, oil, and fats, also these compounds are sensitive to light, heat, oxygen, acids, and alkaline bases. All plants contain carotenoids naturally, which are found in their leaves, fruits, stems, bulbs, and seeds. During fruit ripening, various carotenoids build up in the pericarp and are responsible for the color of the fruits.

The plant pigment can be green, red, brown, orange, salmon and yellow in the fruits and in all plants, bacteria and alga, and these pigments coming because of the variety of the carotenoids. According to (Villa-Rivera and Ochoa-Alejo 2020) the concentration of various carotene changes in the chromoplast of chili peppers as they ripen, contributing to the variety-specific variations in color. The carotenoid concentration in the fruits can change depending on the cultivar, stage of ripening and fruit color according to the harvest time (Lee et al. 1995; Reverte et al. 2000).

The carotenoid is a big group of plant pigments. The carotenoid pigment consists of yellow-colored and red-colored sub-groups. The major carotenoids such as capsanthin, capsorubin, capsanthin 5,6-epoxide, zeaxanthin, and antheraxanthin found in chili peppers are non-provitamin A, while β-carotene and β-cryptoxanthin are the precursors of the vitamin (Hornero-Mendez, 2000). In red-colored chili peppers, capsanthin, capsorubin and cryptocapsin are the dominant carotenoid compounds. Zeaxanthin, lutein, violaxanthin, antheraxanthin are responsible for the yellow color of some chili varieties.

Recent studies have quantitated 34 carotenoids by HPLC (High Performance Liquid Chromatography**)** in red fruits *C. annuum* var. (Deli et al. 2001; Agyemang Duah et al. 2021). Among carotenoids, capsanthin, antheraxanthin, mutatoxanthin, violaxanthin, β--carotene, capsanthin-5,6-epoxide, cucurbitaxanthin, antheraxanthin, mutatoxanthin, violaxanthin, have been detected in chili peppers with different levels depending on the species (Deli et al. 2001; Wahyuni et al. 2011).

In addition to genetic factors preservation technology such as irradiation (Agyemang Duah et al. 2021B) and cooking (Gupta et al. 2006) can impact on the composition of carotenoids in chili

peppers. This explains why even more research have been conducted to understand how these factors can affect the concentration of such compounds and to optimize the technological processes in order to retain the level of the compounds of interest.

The molecule of carotenoid them is out of the tissues is sensitive to some factors as heat, light and oxygen effects and is more prone to degradation and isomerization (Villa-Rivera and Ochoa-Alejo 2020). The carotenoids have antioxidant action, being potentiated by capsanthin and capsorubin (Murakami et al., 2000). The presence of the carbonyl group in the chemical structure capsorubin has two carbonyl groups and has a stronger antioxidant effect than capsanthin causes this potential (Maoka et al., 2001).

Carotenoids have pharmacological actions that improve health, including anti-inflammatory and photo restorative characteristics. According to (Villa-Rivera and Ochoa-Alejo 2020), these supplements have nutritional benefits for maintaining healthy skin, preventing cancer (prostate, stomach, breast, colon, and skin), and treating obesity, retina damage, Alzheimer's disease, atherosclerosis, and diabetes.

2.3.3. Vitamin C

Due to the structure of their molecules, vitamins C and E have very high antioxidant activity, reducing levels of free radicals and quelling peroxidation events in the human body, with the result being a decreased risk of heart disease and several cancers (Navarro et al. 2006). Positive health effects, a variety of spices, including (curcuma, garlic, ginger, chili pepper, turmeric, saffron, and black pepper), have been utilized as food flavoring and medicines (Zheng et al. 2016).

Vitamin C (ascorbic acid) is a water-soluble vitamin that is affected by high temperatures, oxygen, processing methods, postharvest, and storage. Humans require one micronutrient, which must be obtained through dietary sources because the intestinal microflora cannot produce it (Said, 2011), around 90% of the vitamin C comes from fruits and vegetables (Lee and Kader, 2000).

In humans this vitamin participates in various biosynthesis in the body and has numerous benefits such as lowering the risk of cardiovascular disease, aiding in the absorption of iron, preventing colds, and acting as a cofactor in biosynthesis in collagen, carnitine, tyrosine, and peptide hormones (Oslen and Bunge, 1986; Eldridge et al. 1987). Ascorbic acid also participates in the systems and distribution in the brain, central nervous system, neurodegenerative diseases (Alzheimer’s Parkinson’s, Huntington’s, Multiple Sclerosis), Psychiatric disorders (Depression, anxiety, Schizophrenia, Kocot et al. 2017) and oncology diseases (Fritz et al. 2014).

The amount of vitamin C in food is affected by several factors, including species, genotype, maturity stage, storage, and processing. The studies are unclear about vitamin C accumulation and the factors that can influence the amount in fruits.

One studied with *C baccatum* and different genotypes analyzed vitamin C and found the highest amount of the vitamin C and reducing sugars is in mature unripe and the genotypes can influence by the variation (Perla et al. 2016). Four hybrid chili peppers were studied, and the authors discovered a trend in which the ascorbic acid content increases with ripeness, with only one hybrid having the highest level in the intermediate stage (Nagy et al. 2015).

The tendency to increase the acid ascorbic during maturation is relative to the reducing sugars (Perla et al. 2016; Diaz et al. 1998) and this happen because most plants and animals synthesize ascorbic acid from D-glucose or D-galactose, both reducing sugars (Naidu, 2003). The concentration of vitamin C (ascorbic acid and reduced ascorbic acid) in the leaves and fruits varies according to cultivar and ripening stage (Chiaiese et al 2019).

For humans the vitamin C increases the barrier integrity (collagen), metabolic energy (carnitine), hormonal regulation (catecholamines and amidated peptides) and decrease gene transcription (hypoxia-inducible factor), epigenetic regulation (DNA Methylation, and histone methylation) according to (Carr and Maggini 2017).

2.3.4. Vitamin E

Vitamin E is a lipid-soluble vitamin with eight natural isoforms classified as tocopherol and tocotrienol α, β, γ, δ. The number and position of methyl substituents in the head group of the chromanol ring determines the difference between vitamin E compounds. The most common isoform of vitamin E is α-tocopherol, and the majority of vitamin E research is focused on this compound.

Humans do not produce vitamin E, so we must obtain it from our diet. Vitamin E is found in plant oils such as salad oil, nuts, margarines, cereals, and palm oil. According to the literature, vitamin E has antioxidant, anti-inflammatory, anti-proliferative, anti-angiogenic, immune modulatory mechanisms, and inhibits the HMG CoA reductase enzyme (Meganathan and Fu, 2016). The activity of vitamin E with antioxidant activity have been discover by Cummings et al. (1931).

The activity of vitamin E compounds is related to the number and position of methyl substituents in the head group, and this difference provides antioxidant activity and provides protection membrane

lipids in plant cells from oxidative degeneration (Rizvi et al. 2014). The vitamin E in humans' body is powerful neuroprotective, anticancer and cholesterol lowering properties, cardiovascular diseases according to Colombo (2010).

Vitamin E studies in fruits and vegetables have focused on how these compounds change depending on ripening stage, cultivar, irrigation, dry methodology, and biotic and abiotic factors. The literature has already found that the levels of some secondary compounds in plants are related to maturity, and these compounds include carotenoids, tocopherols, and phytosterols (Wahyuni et al. 2013). These metabolites not only act in defense mechanisms that protect the plant from several biotic and abiotic stresses (Schulze and Spiteller 2009; Park et al. 2012a).

Chili pepper is one of several fruits and vegetables that can provide beneficial effects for human health maintenance and disease prevention because it is widely used in cooking. Additionally, more research has been focused on how the level of nutrients and antioxidants are affected by variety, genotype, and cultivar conditions (Marı´n et al., 2004).

Because of the industrial and commercial interests in pepper, research into pepper and vitamin E is expanding. Pepper is also the subject of studies and interests in vitamin E levels in various types of research different variety, dry methodology, irradiation, and fresh chili.

According to the Kim et al. 2017, studies with chili have shown that as the carotenoid content increased, so did the tocopherol content and the phytosterol content. This factor was also observed in the authors' study with different chilies under γ-irradiation (Duah et al. 2021B).

Because plants cannot change their environment when it is unfavorable to them, they must adapt in other ways, one of which is the production of secondary metabolic. Plants develop a complex antioxidant defense mechanism, and vitamin E is one of the compounds that contribute to this defense (Esteban, R. et al. 2009).

According to the Hernandez-Verdeja and Strand 2018; Cardamone et al. 2018; Tikkanen, et al. 2014 the mechanism of antioxidant protections is for preventing reactive oxygen species (ROS) from being adequately counterbalanced and affecting photoinhibition of photosynthesis in plants as well as damaging proteins, lipids, and nucleic acids in the planting (Muñoz and Munné-Bosh 2019). Most studies have focused on α-tocopherol accumulation in photosynthetic tissues, but all tocopherols and tocotrienols exist in non-photosynthetic tissues such as seeds, fruits, flowers, and tubers (Muñoz and Munné-Bosh 2019).

As a result, vitamin E, particularly α-tocopherol, has been shown to play a crucial role in photoprotection when leaves are exposed to photo-oxidative stress, which can be caused either by intense light or a variety of environmental stress factors that lead to excess excitation energy in chloroplasts, such as water stress, acidification, extreme temperatures, or metal toxicity (Havaux et al 2005 and Jin, S., and Daniell, 2014).

2.4. Technological factors

2.4.1. Drying technology

Drying technology is one of the food technologies have been focused on the research for found the best temperature and the best technology for dry chili peppers. Industries have a great interest in developing and optimization the drying technologies that can preserve the compounds of vegetables and fruits and make them with a longer shelf life. A method of preserving is the drying process and has been widely used for good preservation.

The drying process has been used for fruits and vegetables. Drying technology modifies the structural, chemical, organoleptic and nutritional and it occasion the change in the quality of the dry material (Arslan and Özcan 2011). On the other hand the advantages of the technique is to reduce the water content and thus limit the enzymatic action, the microbial degradation and extending the shelf life (Guiné 2018; Palma-Orozco et al. 2021) reducing the storage volume and decreasing transport costs (Govindarajan, 1985).

Color is one of the most important qualities of red pepper, which affects consumers' preferences. (Kin et al. 2006) and is affected by the drying technologies. Even though the drying can have an impact on quality in terms of physical, structural, chemical, and nutrient content, the food industry has been optimizing the drying in chili peppers to maintain the pungency, color, test, aroma, flavors, and major vitamins and compounds. The importance of this technology stems from the fact that chili peppers have a shorter shelf life after harvest.

According to studies cited in the literature, drying technologies can preserve the content of one compound; however, some research finds that higher temperatures around 70 °C preserve the capsaicin better (Palma-Orozco et al. 2021) and low temperature (30 °C and 50 °C) the capsaicin decrease (Chili et al 2019).

The variety of chili pepper can affect the amount of capsaicin in drying chili (González-Zamora et al. 2013) studied dry samples at 65 °C / 72h in eight varieties of *C. annum* and discovered that the quantity of capsaicin is related to the variety. Another factor that catches the eye is the peroxidase

enzyme. Each enzyme in the fruit has one optimum condition for doing the syntheses, and the enzyme peroxidase, which degrades the capsaicin, is inactivated after 16 minutes of heating at 75-80 °C (Bernal, Calderon et al. 1993).

The drying process at a higher temperature is one method for maintaining and conserving carotenoids as well as bioactivities (Loizzo et al. 2013). Chili pepper drying at 70 °C has a higher concentration of all carotenoids, but low temperatures (30 °C and 50 °C) did not preserve the total carotenoids (Chili et al. 2019). Therefore, the low temperature the biosynthesis and the degradation of the carotenoids make one decrease in the concentration (Kevreˇsan et al., 2009; Topuz et al., 2009) the dry process with

In general, some scientists found that the dry methodology maintains the levels of vitamins and bioactivities (Loizzo et al. 2013), while the others found that the carotenoid and free and esterified carotenoids decrease by nearly 50% (Campos-Hernández et al. 2018; Kevrešan et al. 2009). So according to research, a higher temperature below 70 °F can actually affect the carotenoids in chili pepper (pungency or non-pungency) (Daood et al. 2006) A same author also looked into the various carotenoid's compounds, mono-esters and di-esters, and found that while each compound has a different thermal stability, they all tend to decrease at higher temperatures. For drying technology in chili pepper needs to be attentive to the variety, according to this carotenoid component can behave differently (Daood et al. 2006; Minguez-Mosquera et al. 1994).

The stability of carotenoids in chili pepper during storage depends on the drying conditions and the rate of deterioration increasing as the drying temperature increases (Doymaz and Pala, 2002; Vega-Galvez et al., 2008). The drying process can have a greater impact on the content of phenolic compounds, as an increase in drying temperature has a significant effect on the overall phenolic content, and phenol content decreased at all temperatures (Vega-Gálvez et al. 2009).

Ascorbic acid is used as an antioxidant in plant physiological systems and is thus the first oxidation barrier in paprika during drying and storage (Daood et al. 2006) and temperatures above 70 °C, the reduction of acid ascorbic can be more than 50% (Daood et al. 2006). Because the molecule of vitamin C is unstable when exposed to light, oxygen, and heat, the acid ascorbic is used as an indicator of the quality of food processing (Podsedek, 2007; Vega-Gálvez et al. 2009).

Chili pepper was examined under various drying and storage conditions, and all ascorbic acid content was significantly reduced in all conditions. (Kim et al. 2006; Daood et al. 1996) investigated fresh and dried chili pepper (natural dried and forced air-dried) and found a significant decrease in

dried samples. Storage is another step that has a major impact on the content of ascorbic acid and α-tocopherol and was studied (Daood et al. 1996).

The effect of the drying process and the ascorbic acid have been studying from different authors and methodology in different chili peppers varieties (Romauli et al. 2021; Agyemang Duah et al. 2021 this news researches the main found is one decrease in ascorbic acid and the dry process can affect in some way.

Chili peppers drying in the sun is a very traditional technique. (Oni, 2015) and powder chili are extensively used as a spice in as a main component of various globally dishes, as well as one of the techniques for storing for a long period of time. Different parameters in the dry process can influence the results, including the variety, the geometry of the sample (whole fruit, slices, cubes, cut in the middle), temperature (natural from the sun, or artificial), and the time that the sample will be in the drying process Kim et al. 2004.

The drying technologies efficiency is dependent on heat and mass transfer during the drying process, as well as the final moisture of the product (Chili et al. 2019). As a result, the estimated water diffusivity and the correct pepper slice size are the most important characteristics to predict the pepper's drying dynamics and thermal efficiency (Chili et al. 2019). In the literature, more articles refer to the ring process in *C. annum* L. for the others domestic species (*C. chinense, C. frutescens, C.baccatum and C. pubescens*) have less studied.

2.4.2. Genetic factor

The agricultural challenge in all productions is really to select cultivars that have been disease-resistant, more adaptable to biotic and abiotic stress, and start producing fruits and vegetables with striking quality and flavors quality. Hence it is necessary to study, how the genotype factor contributed most to the variability in production components (Barchenger et al. 2018). Create crop varieties that are better adapted to recurrent climate changes, as well as varieties that maximize sustainability and production (Causse et al. 2020).

According to the area the variety of chili pepper can have different phenotypic. Zhang et al. (2016) studied chili peppers in different provinces and found that genetic variations are possibly shaped by the genetic conditions of each region or are a consequence of the evolution of the farmer to the region destined to choose the best seed with the best traits for production and consumption. Further, the research (Zhang et al. 2016) reveals the impact of human (farmer) selection in primary and secondary centers of diversification, driven by local adaptation but also consumption.

Most studies do not mention or study the plant's genetics, instead focusing on the effect of compounds, vitamins, and yield. The interaction between the genotype and the environment influences how the plant responds to environmental changes (Gurung et al. 2012) studies also detect the environmental effect on how the plant produces carotenoid and flavonoid (Lee et al. 2005).

Indeed, climate change and the risk of resistance breakdown affect disease resistance durability; thereby, there is an urgent need to develop new resistant varieties that can be adapted to a variety of pedoclimatic conditions. Gene pyramiding strategies, in this context, can allow the accumulation of resistance genes in a single genotype, resulting in more durable and broad-spectrum mechanisms (Özkaynak et al. 2014). The strategy can be implemented by combining one or more significant gene alleles (Tan et al. 2010).

2.4.3. Abiotic stress

With growing concerns about climatic uncertainties, abiotic stresses have emerged as the most serious threat to agricultural production worldwide (Bray et al. 2000). Among several works with *Capsicum* spp. and other crops, one factor that cannot be controlled in the open field is abiotic factors. Several studies have shown that the result of the compounds in the fruits is affected by abiotic factors such as geographic location, chili variety, and harvest time.

The plant's biological system responds differently to abiotic factors (heat, cold, salinity, and osmotic stress), and the plant has different expression profiles in reaction to abiotic stresses (Kang et al. 2020). This research shows that each abiotic stress causes a distinct response in plant morphological, physiological, and biochemical compounds.

There are two reasons to study abiotic factors (1) Industry has been developing crop production technologies with variety resistance to environmental stress and good fruit quality (Jimenez-Garcia et al. 2014); (2) global climate causing changes in the environmental scenario, will also affect crop production directly. Due to this potential change in the global scenario, research findings on the interaction of abiotic factors in food production have increase (Fedoroff et al., 2010).

Research with the same variety in different accessions, the chili peppers react differently depending on the environment of each location. (Meckelmann et al. 2015) reported in their study that the same varieties were planted in different accession and location, demonstrating that these two factors and the growing conditions can have a significant interaction in chili peppers. Such research is very interesting for traits that facilitate the selection of accessories for special needs and consumer expectations, according to (Meckelmann et al. 2015).

Several parameters have been considered within breeding programs aimed at the formation of varieties preferable for productivity and quality characteristics for consumers; as a result, many studies on the influence of abiotic stresses on crop yields have been conducted.

Water scarcity is one of the main limitations to crop productivity worldwide, impacting plant biosynthesis and, therefore, turgor pressure, in addition to growth, yield, pigment content, and photosynthetic activity (Anjum et al. 2011). When there is a water shortage in the soil, the plant must adapt to the water stress conditions.

Even though water is essential for crop production, research into WS has increased to determine how much water can be inferred in crop production and to implement the new irrigation system. The goal of deficit irrigation is to improve crop water use efficiency (WUE) by reducing the amount of water used in watering or the number of irrigation events (Kirda, 2002).

Drought stress can have an influence on the biochemical response of the chili pepper. The chili can be more pungency underwater deficit due to an increase in capsaicin (Sung et al. 2005) and (Phimchan et al. 2012) and an increase in dihydrocapsaicin (Sung et al. 2005).

Nonetheless, photosynthetic pigments such as chlorophyl and carotenoids, as well as soluble sugars and carbohydrates, can increase in response to water stress. (El-Ghinbihi and Hassan 2007; and Klar et al. 2006). Deficiency of water also can impact yield per hectare; when the chili crop is under deficit, the yield can be lower compared to full irrigation; on the other hand, a profit-maximizing strategy requires substantially less water than a maximum yield strategy (Ali et al. 2007; Yang et al. 2018).

The water stress conduct by Khan et al. 2008; Khan et al. 2009 demonstrate the relation between the deficit of water (or drought stress response) and the parameters and yield in chili papers and showed the stress effects either due to excess or deficit moisture on the parameters studied. drought stress response.

Moreover, determining the local and regional sensitivity of chili peppers to water stress at various growth stages must help growers adopt improved irrigation schedules to achieve better yields and quality of the fruits in conjunction with less water consumption (Yang et al. 2018).

The soil is another abiotic factor that affects crop production. According to research, different regions have distinct soil characteristics, and this abiotic factor can influence crop production. It is critical to conduct research on the influence of soil in different regions to choose the best variety for the region and soil.

Abiotic factors (soil, ripening, harvest cycle) contribution of each chemical and physical characteristics of the fruits according to Oney Mantolvo et al. 2021, the ripening stages this research found the environmental factors and type of soil impact more in the fruits than the ripening stage, the author. The plant soil feedback (PSF) was studied from (Nuske et al. 2021). Taken together, these findings demonstrate that biotic and abiotic soil effects are both important components that can influence the plants growing.

2.4.4. Temperature

Previous research found that the ascorbate content in pre-harvest sweet pepper fruits was involved in the responses to temperature changes. Pepper growth at higher temperatures had a higher reduced ascorbate content than pepper growth at temperatures lower (Mateos et al 2013). Because of this, the air temperature will also influence crop production, and when we think of crop production in open fields in Hungary, we must consider the temperature during chili pepper crop production. According to this viewpoint, Guy CL (1990) demonstrates in your research that crop production when exposed to freezing environments can cause serious damage to plant cells because ice formation destroys and malfunctions cellular membranes.

Cold and heat conditions have been studied in various plants, and the authors discovered that cold temperature conditions modify the metabolism of the plant and change the in *Arabidopsis* sp. Furthermore, different compound was discovered in the plant metabolism during cold acclimation (Obata and Ferne 2012; Espinoza et al. 2010).

Air temperature is a challenge in agricultural production that has an impact on agriculture all over the world. Under this factor, researchers have thoroughly investigated scenarios of climate change in food crop production around the world, such as the effects on crop productivity in Africa, West Africa, South Asia, and Italy (Knox et al. 2012, Bandara and Cai 2014; Bocchiola et al. 2013).

Additionally, increased temperatures may have an indirect or direct impact on agricultural productivity yield, in the grown, morphological, fruit quality, diameter, and weight, and phytochemicals (Gunawardena and De Silva, 2014). The amount of capsaicin in the chili pepper can also greatly increase as the temperature rises (Rahman et al. 2012). This correlation might also affect the amount of capsaicin in chili peppers in various territories with differing temperatures.

3. MATERIALS AND METHODS

3.1. Experimental conditions

The research was conducted at the Horticulture Institute experimental field, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary (latitude 47°61′ N, long. 19°32′ E) with annual average precipitation of around 560 mm. The soil texture was characterized as sandy loam, mostly cambisols with 65% of sand, 8% of clay, 27% of silt fraction, and 1.6% organic matter. The soil had a slight to moderately alkaline pH of 7.9, 16% field capacity, and bulk density of 1.54 g m^{-3} when a depth of about 35 cm of the upper layer of the soil was considered. Chili pepper cultivars 'Hetényi Parázs' (HET), 'Unikal' (UNIK), 'Unijol' (UNIJ), and Habanero (HAB) seedlings were obtained from Univer Product Zrt, the leading food industry in Hungary.

After 40 days of germination in a nursery, the seedlings were transported for open field cultivation on May 13, 2019, and May 14, 2020, each season against three (3) different WS treatments; 0% (control except for natural precipitation), 50% deficit irrigation and 100% optimum WS. The seedlings were cultivated in twin rows with 0.25 m spacing inside the rows and 0.25 m between plants in a row, with a plant density of 6.66 plants m^{-2} for HET and UNIK. In the case of UNIJ and HAB, the seedlings were planted with a spacing of 0.5 m inside the rows and 0.5 m between plants in a row, with a plant density of 3.33 plants m^{-2}. The spacing between adjacent twin rows of all cultivars was 0.75 m in 2019 and 1.5 m in 2020. The adjusted spacing between twin rows in 2019 was purposely done to manage weed growth easily. The entire experiment was arranged in a randomised complete block design (RCBD) with four replicates or blocks per treatment on a one-hectare plot of land.

3.2. Irrigation system and management

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., 2018). The trends of mean temperature and precipitation in 2019 was 20.25°C and 132.6 mm and in 2020 was 18.92 and 478.6 mm (Table 1 and Figure. 1). At each experimental season, weather predictions by the Hungarian Meteorological Services from a nearby station were taken into consideration. The daily minimum and maximum meteorological variables—

temperature, relative humidity, and precipitation were calculated. 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%).

Table 1: Meteorological record and WS throughout the chili pepper growing seasons.

Year	Mean temperature (°C)	Mean relative humidity (%)	Precipitation and rainfall (mm)	Irrigation (mm)		Total water received by plants (mm)[1]		
				50%	100%	0%	50%	100%
2019	20.3	72.3	132.6	152.2	289.0	132.6	284.8	421.6
2020	18.9	74.6	478.6	94.2	186.5	478.6	572.8	665.1

[1]0%, control; 50%, deficit irrigation; 100%, optimum WS

During the heavy rainfall period, the crop coefficient (Kc) guidance was considered, and regular irrigation was paused. Regular irrigation of plants was resumed 5 or 6 days after the rains. Generally, irrigation of plants was done two times per week depending on precipitation, and once a week, plants received uniform fertilization in the form of granulates proportion of nitrogen (NO_3), phosphorus (P_2O_5), and potassium (K_2O) YaraMila Complex 12–11–18 + 20% sulphur (SO_3) (Yara & Co., Veszprem, Hungary).

During the plant growth periods (2019 and 2020), healthy and newly emerged plant leaves were randomly selected in replicates for relative chlorophyll content (expressed as SPAD values), leaf chlorophyll fluorescence (Fv/Fm), and canopy temperature (°C) measurements. Harvested peppers of high-quality yield were collected for the total weight of marketable fruits expressed as tons per hectare. During the harvest, the fruits' weight was measured, and the yield per hectare was calculated from these data. Fully ripened and healthy fruits in the same replication were randomly selected for phytochemical analyses (vitamin C, capsaicinoids, carotenoids, and vitamin E/ tocopherols) and further analyses of irradiated peppers.

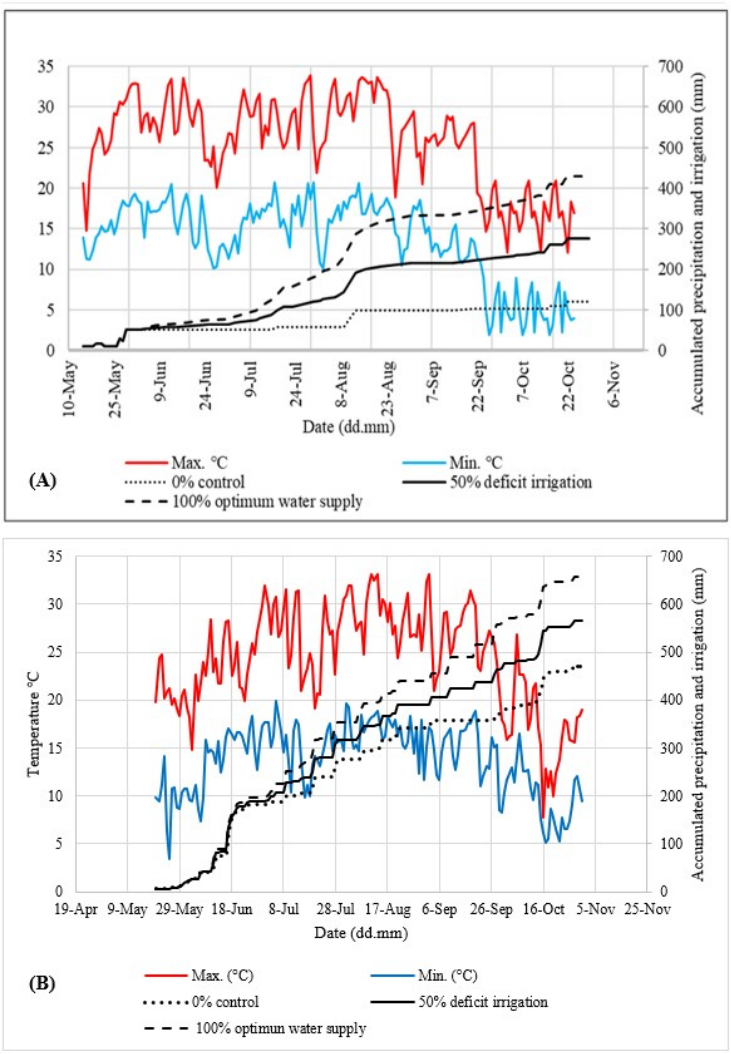

Figure 1: *Trends of daily maximum (Max.) and minimum (Min.) air temperature (°C), and accumulated precipitation and irrigation (mm) for the growing seasons, (A) 2019 (B) 2020.*

3.3. Plant materials

Chili pepper cultivars 'Hetényi Parázs' (HET), 'Unikal' (UNIK), 'Unijol' (UNIJ), and Habanero (HAB) seedlings were obtained from Univer Product Zrt, the leading food industry in Hungary.

Hetényi Parázs

Hetényi Parázs F1 is characterised by outstanding yield and content. It has the highest dry matter and capsaicin content among the hot pepper varieties. It has *Xanthomonas* bacteria- (HR: Tm0,1,2) as well as tobacco mosaic virus resistance (Xcv: 0-3,7,8). It is mainly recommended for intensive cultivation, and it is early ripening.

Unikal

The Unikal cultivar has Xanthomonas bacterial resistance (HR: Xcv: 0-3,7,8) and is less susceptible to cucumber mosaic virus (CMV). Its content values are like the Unijol variety. The content of capsaicin is ~200 mg/kg. It is capable of high yields, suitable for both replanting and transplanting. In terms of ripening, it is medium-late ripening (Univer Product ZRt 2018).

Unijol

Unijol F1 is an indeterminate and interspecific hybrid (*C. annuum* × *C. chinense*). It grows a bush with strong growth, twice the size of traditional peppers. It contains the Bs2 gene, which confers resistance to the bacterium *Xanthomona*s. The average berry weight of the plant is around 10 g. The dry matter content is slightly lower than the average dry matter content of 18% for sweet peppers, approximately 15-16% for this hybrid. The capsaicin content is very high, about 12,000 mg/kg on a dry weight basis. (Timár et al., 2016).

Habanero

The Habanero variety belongs to the *C. chinense* pepper species. The berry weight is 8-10 g on average and has a width of 2.5 and a length of 6.4 cm. Its dry matter content is between 10-12%. The capsaicinoid content varies between 10,000 and 15,000 mg/kg on a dry weight basis. As the fruit ripens, it turns green and then orange. (Bosland and Votava 2012).

3.4. Physiological responses

3.4.1. Relative chlorophyll content (SPAD value)

At the time of flowering and harvesting of the peppers, the SPAD index was determined using a chlorophyll meter SPAD–502 (Konica Minolta, Warrington, UK) in fully expanded leaf from the apex to the plant base. The chlorophyll meter SPAD–502, a non-destructive device, measures transmittance to determine leaves' greenness (Jifon et al, 2005). The device measures the relative chlorophyll content of a plant leaf based on the absorbance of 650 nm wavelengths of light, using as a reference the 940 nm wavelength infrared light. During the measurement, the instrument calculates the SPAD value from the intensity of the infrared and red light passing through the leaf, which shows a close correlation with the chlorophyll content measured by an accurate analytical method (Madeira et al., 2003; Del Amor, 2006; Xiong et al., 2015.)

Four plants were randomly selected per block, and in each plant, four leaves were measured. In all, sixteen leaves per treatment of all cultivars were measured. The SPAD–502 chlorophyll meter was calibrated before every measurement.

3.4.2. Canopy temperature

Canopy temperature reflects the physiological activity of plants, and their growth can be monitored by measurement. Raytek infrared remote thermometer (Raytek Corporation, Santa Cruz, CA, USA) was used in this experiment. This portable battery-powered instrument can measure the surface temperature of objects. Its operating principle, which can measure 99% of the energy emitted by the object in the field of view of the telemetry unit with an error of ± 1%, makes it possible to determine plants' leaf temperature. In all blocks, ten plant canopy per treatment of all cultivars were randomly selected in this experiment, and the temperature was recorded. No calibration is required before using the instrument; however, environmental factors, especially clouds, were considered while using the instrument.

3.4.3. Chlorophyll fluorescence

Chlorophyll fluorescence measures the physiological health of plants and indicates a stress response. A portable PAM 2500 fluorometer (Heinz Walz GmbH, Effeltrich, Germany) was used to measure chlorophyll fluorescence in this experiment. Measurement was done weekly on sunny days at noon during the entire study period. Four fully developed top leaves of a single plant from each replicate were affixed with leaf clips for a 35 min dark adaption before fluorescence was measured. The Fv/Fm ratio, the maximum quantum efficiency of PSII was quantified and determined by the fast kinetics method in the PamWin 3.0 software (Van Goethen et al. 2013).

Chlorophyll fluorescence equation: $Fv/Fm = (Fm–Fo)/Fm$,

where Fo = initial fluorescence

Fm = maximal fluorescence

Fv = variable fluorescence (Fm–Fo).

3.4.4. Soil moisture

Soil moisture generally refers to the amount of water stored in the spaces (pores) between soil particles using PT-1 soil moisture digital spear (Kapacitív KKT, Budapest, Hungary). During measurements, natural precipitation and fertigation were taken into consideration, focusing on the unsaturated soil zone. Three different rows were randomly selected for soil moisture measurement.

3.4.5. Yield

The total production of fruits per plant was obtained by manually harvest between August and October in each year (Table 2). Average fruit weight was measured using a weighing scale of 0.01 g precision analytical standard balance (Mettler-Toledo Kft. Budapest, Hungary). Four successive harvests were done between August and October each year until the frost began.

Table 2: The date of planting and harvesting for the two experimental years.

Year	Planting date	Harvest date
2019	13 May	13 August
		10 September
		07 October
		28 October
2020	14 May	
		09 September
		30 September
		28 October

3.5. Chromatographic Analyses

3.5.1. Chemicals used in the analytics.

All analytical grade solvents and chemicals, as well as High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) grade organic solvents used in the analyses, were purchased from VWR (Debrecen, Hungary). Standard capsaicin 95% (CAP), nor-dihydrocapsaicin 95% (NDC) and dihydrocapsaicin 85 % (DC), zeaxanthin 95%, β-carotene 93%, 8- β-apo-carotenal 96%, D-α-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).

3.5.2. Extraction and HPLC determination of capsaicinoids

Individual and total Capsaicinoid concentration (nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin derivatives, and homodihydro-capsaicin derivatives) that appeared on the chromatogram was determined and calculated following the method of (Daood et al. 2015).

About 3 grams of homogenized pepper fruit without seeds were crushed in a crucible mortar with quartz sand. 50 mL of analytical-grade methanol was gradually added before the mixture was

carefully transferred to a 100 mL Erlenmeyer flask with a stopper. The mixture was subjected to ultrasonication in an ultrasonic bath device for 3 minutes and then filtered through a filter paper. The filtrate was then 10 times diluted for 'Hetényi Parázs' and 'Unikal' and 50 times for Unijol and Habanero with chromatography grade methanol and purified through a 0.22 µm PTFE (Chromfilter) syringe into 1.5 mL vails for injection onto the HPLC column.

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) (Appendix 1)

3.5.3. Extraction and HPLC determination of vitamin C

Vitamin C content was determined according to the method and HPLC protocols of Nagy et al. (2015). About 3 grams of homogenized pepper fruit (seed excluded) was crushed in a crucible mortar with quartz sand. Then 30 mL of 3% metaphosphoric acid solution was gradually added to the mixture and transferred to a 100 mL Erlenmeyer flask with a stopper. The mixture was subjected to ultrasonication for 2 min, and mechanical shaking for 20 min. The mixture was filtered through a filter paper and further purified by passing it through a 0.45 mm cellulose acetate (Whatman) syringe filter before it was injected into an HPLC column. For the quantitative determination of ascorbic acid, sample data were compared to that generated using standard materials (Sigma-Aldrich, Budapest, Hungary). In case of dried pepper samples, the pepper was ground by a coffee mill to pass a 20-mesh sieve and 1 gram was immediately taken and extracted with 30 mL of methanol and further analyzed as described for fresh chili sample.

Metaphosphoric acid solution was prepared by dissolving 30 grams of metaphosphoric acid crystals into 1 L of distilled water following by mechanical shaking till complete solubility is achieved.

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.

3.5.4. Extraction and determination of carotenoids and tocopherols

Carotenoids and tocopherols were determined according to the separation protocols of Daood et al. (2014). From a well-homogenized pepper fruit sample 2.5 grams (seed excluded) of 'Hetényi Parázs', 'Unikal', 'Unijol', and 3.5 grams of 'Habanero' were taken for the analysis of fat-soluble carotenoids and tocopherols. The sample was crushed in a crucible mortar with quartz sand., and 20 mL of methanol were added and kept for 1-2 minutes. The supernatant (methanol) was decanted into a 100mL Erlenmeyer flask with stopper. The carotenoids and tocopherols were extracted by stepwise an addition of 60 mL of 10:50 methanol-di-chloroethane to the residues in the crucible. The extract was collected with the methanol fraction in the flask. The two different phases were separated by addition of 1 mL HPLC grade water. The less polar containing carotenoids and tocopherols was separated in separatory funnel and the organic solvent was dropped into a round-bottom flask through anhydrous sodium sulphate placed in a glass funnel containing filter paper. The extract was then evaporated to dryness using vacuum-controlled rotary evaporator at 40 °C. The residues were redissolved first in 5 mL of a mixture of 10:35:55 methanol- acetonitrile-isopropanol followed by 5 mL HPLC grade methanol to ensure complete solubilization of the extracted materials. Before injection. the filtrate was further cleaned up by passing through a 0.22 µm PTFE membrane syringe filter into 1.5 mL vails and injected into the HPLC column.

In the case of dry chili peppers, 0.5 grams of ground sample were extracted with 50 mL of a mixture of 1:1:2 methanol-acetone-dichloroethane with mechanical shaking for 15 min and 4 min ultrasonication using a water bath ultrasonic device. The mixture was filtered through a filter paper and passed to a round-bottom flask for solvent evaporation. The subsequent steps were as earlier mentioned for fresh pepper samples.

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 the tandem mass spectrometry (MS/MS) detection and for the optimization of the electrospray ionization (ESI) source parameters, flow injection analysis (FIA) of all-trans- β-carotene standard was used. All experiments were conducted with positive ionization mode with the following settings: the capillary voltage was 1.5 kV, nebulizer gas 7 bar, desolation temperature 400 °C, cone gas flow 200 L/h, desolation gas flow 800 L/h, source temperature 150°C. Since a number of different unknown compounds was expected, a cone voltage ramp was applied between 30 and 75 V, where the gradient was 0.15 V/Da. Quadrupoles were set to unit resolution, while for collision gas, argon 5.0 was used with 0.15 ml/min. For collision energy setting, a ramp was applied from 5 to 60 eV, and the gradient was 0.061 V/Da. Soft transmission mode was enabled during experiments in the step-wave apparatus of the instrument to reduce the possibility of in-source fragmentation effects before the first quadrupole.

After chromatography and DAD detection, 10 µl/minute of methanol containing 1 % formic acid was combined via infusion with the flow towards the ESI source of the mass spectrometer with a syringe pump to enhance the formation of (M+H) +ions. Moreover, most carotenoids form an M+ radical ion, so (M+H) + form was only enhanced to have additional confirmation for the parent masses of carotenoids since the peaks were identified based on comparison of their spectral characteristics and retention times with those of literature data (Schweiggert et al. 2005). In addition, LC-DAD-MS/MS method was used to emphasize the molecular ion mass (m/z) for each compound and fragmentation of the unidentified carotenoids. *Cis*-isomers were characterized by the appearance of an extra absorption maximum between 340 and 362 nm and the value of Q-ratio (Lin and Chen, 2003; Schieber and Carle, 2005).

Quantitative determination was performed by integration of each peak area at the maximum absorption wavelength provided by DAD and relating it to that of the internal standard (β-8'-apo-carotenal), which was spiked to the samples at known concentration before extraction. In addition, available standard lutein, β-carotene, and all trans-lycopene were used as external standards to emphasize their quantification.

As for tocopherols, the peaks were identified by comparing the retention times with those of the standard materials [γ-tocopherol (γ-toc), β-tocopherol (β-toc), α-tocopherol hydroquinone (α-toc QH2), α-tocopherol (α-toc), and α-tocopherol ester (α-toc ester)]. In the case of tocopherol ester, the extract was also saponified by 30% methanolic KOH to remove the fatty or acetate moiety. The disappearance of the peaks confirmed the ester form of tocopherols.

3.5.5. HPLC instruments

A Hitachi Chromaster HPLC apparatus with a Model 5440 diode-array detector, a Model 5440 Fluorescent detector, a Model 5210 autosampler, and a Model 5110 gradient pump was used in the determination of capsaicinoids, vitamin C, tocopherols, and carotenoids.

The LC-DAD-MS/MS identification of carotenoids was performed using a Waters Acquity I-class UPLC system connected to a Waters Xevo TQ-XS MS/MS.

3.5.6. Dry processing of chili

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.

3.5.7. Statistical analysis

Data were expressed as the mean ± 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 (0%, 50%, and 100%) on physiological responses (SPAD, chlorophyll fluorescence, and canopy temperature) and two-way ANOVA for vitamin C, capsaicinoids (NDC, CAP, DC, HCAP, iDC, and HDCs), tocopherols (γ-toc, β-toc, α-toc QH2, α-toc, and α-toc ester) and carotenoids (free caps, free zeax, caps ME, zeax ME, β-carotene, caps DE and zeax DE). ANOVA was also used to examine significant differences among cultivars (HET, UNIK, UNIJ, and HAB), WS (0%, 50%, and 100%) and harvest periods (1st harvest, 2nd harvest, 3rd harvest, and 4th harvest). In the case of a significant result of the ANOVA, the groups with significant differences were determined by Tukey HSD (Honestly Significant Difference) posthoc test. The average mean yield was calculated for the four harvesting periods per year using Microsoft Excel 2016. All statistical analyses were carried out with IBM SPSS Software package version 25.0 for Windows, at the significance level $\alpha = 0.05$ throughout the study.

4. RESULTS AND DISCUSSION

4.1. Effect of WS on physiological factors during growth of chili pepper

During the 2019 and 2020 cultivation seasons, the various cultivars (HET, UNIK, UNIJ, and HAB) were subjected to three different WS treatments (0% control, 50% water deficit, and 100% optimum WS) before measurement of physiological factors irrigation was done every two (2) weeks in the first year and every week in the second year.

4.1.1. Relative Chlorophyll content (SPAD values)

Similarly, all cultivars in 2019 (Fig. 2A) had significant differences among them. There was no significant effect on WS in the HET cultivar ($F=0.547$, $p=0.582$). UNIK recorded significantly ($p<0.001$) the highest SPAD values. Under 100% conditions, UNIK recorded significantly lower SPAD values. As the WS increased, SPAD content decreased in UNIJ. Also, in HAB, a decrease in SPAD values as the WS increased was detected. However, HAB cultivars that were given 50% treatment were not significantly different from 100% ($F=17.081$, $p<0.001$). Peppers irrigated (100%) recorded the lowest SPAD values and in the non-irrigated ones (0%) the highest. 50 % was significantly higher when compared to 100%.

The WS treatment in cultivars differs substantially from 2020 SPAD values (Fig. 2B). The HET and UNIK have the highest percentages of 100% WS, with respective contents of 79.07 ± 12.40 and 73.47 ± 11.07. The 100% WS had the lowest value from SPAD values for the cultivar UNIJ and HAB (57.25 ± 8.82 and 52.24 ± 5.48, respectively). The cultivars HET and UNIK chlorophyll content in the months of July, August, September, and October did not differ ($p>0.005$) from one another in terms of SPAD values or WS treatment throughout these months. The 100% WS for UNIJ had the lowest value for the entire month, and in September there was no difference ($p>0.005$) in the WS treatment. Also, in July no difference ($p>0.005$) was handled by HAB. In both cultivar HET and UNIK have the highest SPAD values during the months for all WS treatment.

The chlorophyll content indicates the chloroplast development, photosynthetic capacity, leaf nitrogen con- tent or general plant health in both years of research 2019 and 2020 the UNIJ and HAB variety have the lowest value from SPAD because of that we can infer that the chlorophyll content is dependent on the variety. The SAPD could be affected by factors as cultivar, year, growth stages, leaf thickness, leaf positions and the measurement point on the leaf. (Ata-Ul-Karim et al., 2014; Hu et al., 2014).

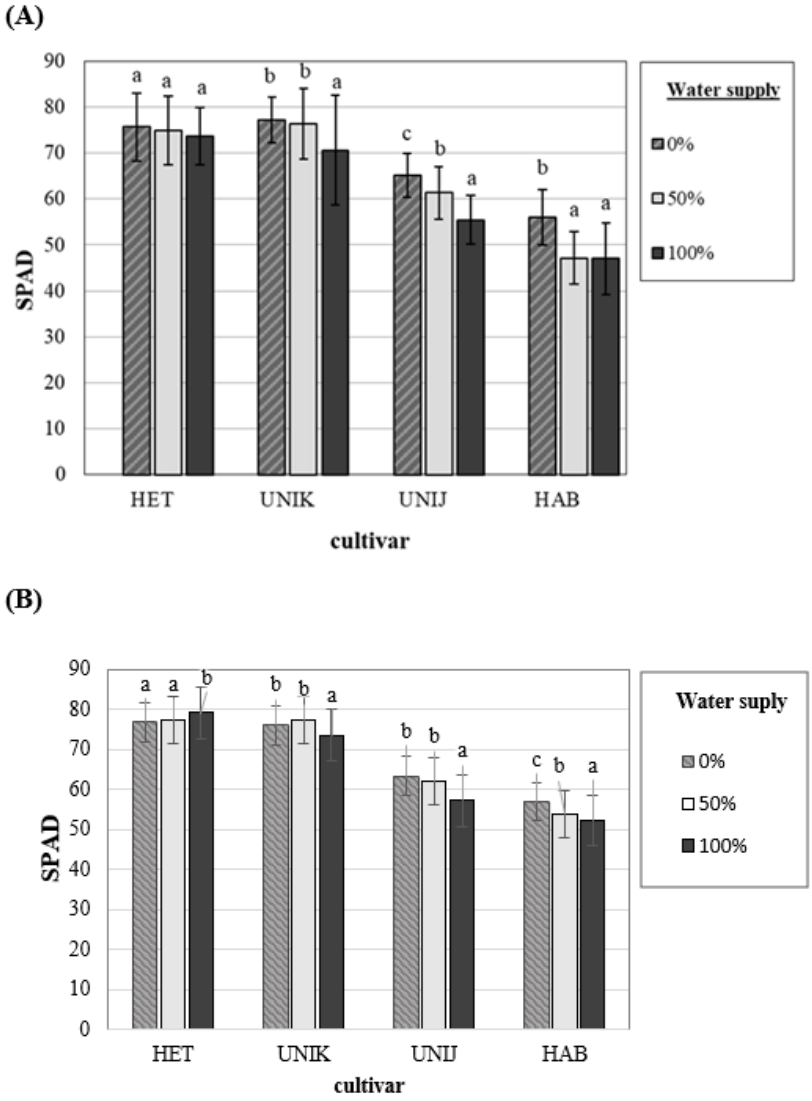

Figure 2: Effect of seasonal WS treatments and cultivar response to relative chlorophyll content expressed as SPAD values in 2019 **(A)** and 2020 **(B).** 0%, control; 50%, deficit irrigation; 100%, optimum WS; HET, Hetényi Parázs; UNIK, Unikal; UNIJ, Unijol; HAB, Habanero.

4.1.2. Canopy temperature

Determination of leaf surface temperature is one of the best indirect methods for estimation of water. WS had no influence on canopy temperature in UNIK, UNIJ, and HAB (Fig 2). Notwithstanding, as WS increased, canopy temperature increased in UNIJ, but on the contrary, that of HAB decreased as WS increased even though there were no significant differences between them.

During the 2020 season, the canopy temperature was effective by the cultivar and the WS (Fig 2B). The highest value of canopy temperature occurred in the 0% WS for the treatment HET (21.46 ± 6.29), UNIK (20.81 ± 6.29) and HAB (21.51 ± 6.68), and for the cultivar UNIJ the highest was

found with 50% (20.57 ± 6.29). The canopy temperature and WS treatment have no difference response during the months July, August, September and October for the cultivars HET, UNIK and UNIJ. For HAB the difference occurred only in July and the lowest effect was in 100% WS.

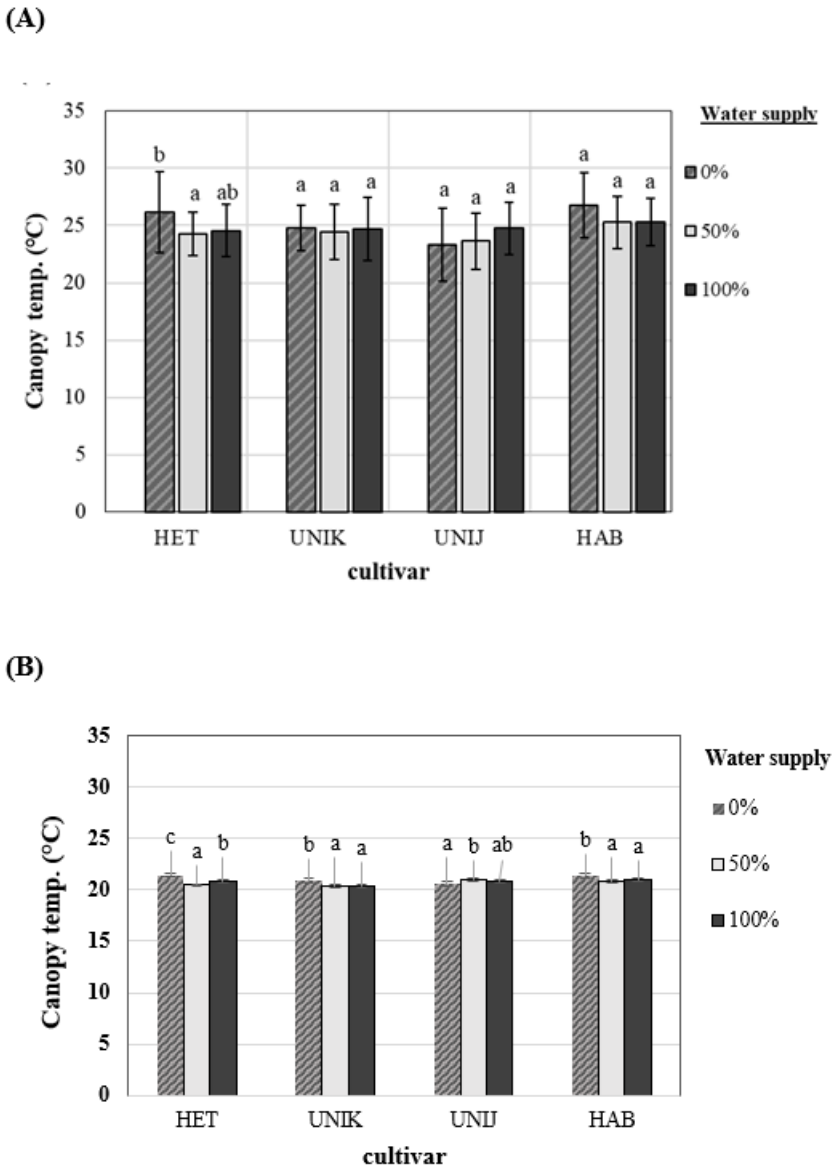

Figure 3: Effect of seasonal WSTs and cultivars' response to canopy temperature under unirrigated and non-irrigated conditions in 2019 **(A)** and 2020 **(B)**. 0%, control; 50%, deficit irrigation; 100%, optimum WS; HET, Hetényi Parázs; UNIK, Unikal; UNIJ, Unijol; HAB, Habanero.

It is important to determine that air temperature is different from the leaf temperature and even though in both year the cultivar was the same and the climate was difference. According to (Chaves, 2013) the instrument used to measure leaf temperature has some limitations because the result depends on microclimate and the rapid changes in environmental conditions, for example on cloudy days, demonstrate high variability of the results. Also, the inter-dependencies between leaf

properties (leaf size and temperature), physiological parameters (leaf conductance, assimilation and transpiration rate) and local environmental variables (air temperature, air humidity, atmospheric CO_2, wind speed, solar and environmental irradiation) (Konrad et al. 2021).

4.1.3. Effect on chlorophyll fluorescence

In the first growing season (Fig. 4A), HET had significantly ($p=0.021$) lower Fv/Fm values with 100% WS. However, there was no significant difference between 100% and 50% ($p<0.001$). Among the other cultivars (UNIK, UNIJ, and HAB), Fv/Fm values of WS treatments were not significantly different from each other. Nonetheless, it was detected that as the WS in HAB increased, Fv/Fm values decreased even though there were no significant differences among them ($p=0.085$).

The leaf chlorophyll fluorescence expressed as the maximum quantum efficiency of PSII (Fv/Fm) was not significantly affected when measured in HET, UNIK, and UNIJ cultivars (fig 11) but were significantly different from HAB peppers. There was no variation in light absorption by the pepper plants. Demmig-Adams et al. (1995) indicated that excess light absorption or light stress during plant leaf growth affects their response to high photon flux densities and PS II efficiency. Light stress or low light absorption was observed in apple leaves resulting in low electron transport in leaves with low nitrogen content (Cheng et al. 2000).

In 2020, none of the chili treatments' measurement by the quantum efficiency of PSII (Fv/Fm) was affected by the irrigation treatments (Fig. 4B). The highest results were obtained in HET (0.73± 0.08), UNIK (0.73± 0.08), UNIJ (0.70± 0.13), and HAB (0.72± 0.08). Only the water treatment for HET in the month of August showed a discernible change, according to the findings of each treatment organized by month. There was no variation in light absorption by the pepper plants between 0% and 50%, and both values were more than the treatment 100%. (0.75±0.03). The other treatments, UNIK, UNIJ, and HAB, showed no appreciable variation efficiency of PSII (Fv/Fm) during the measurement months.

In both year of experiment even though the chlorophyll fluorescence was not affected by the WS for all cultivars HAB showed the lowest value.

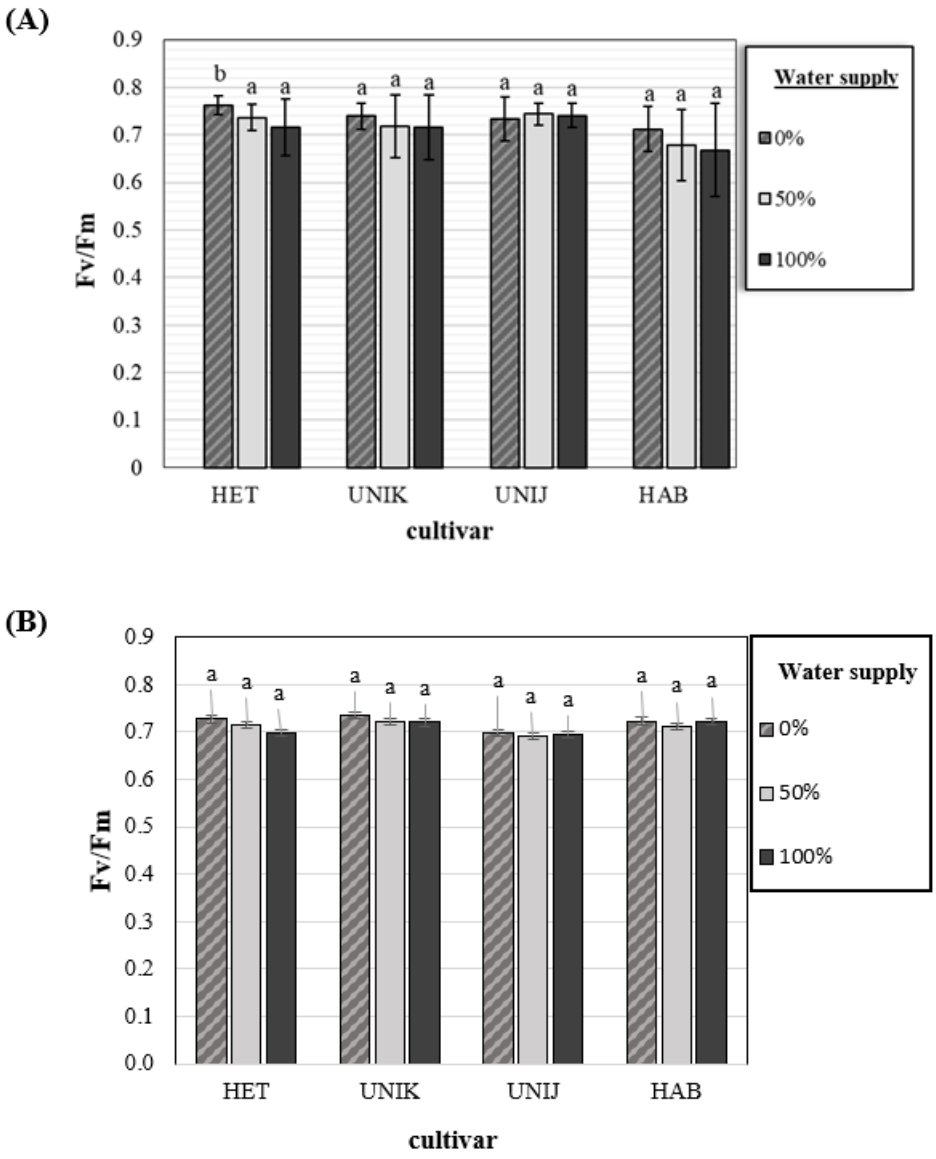

Figure 4 Effect of WS treatments on chlorophyll fluorescence (Fv/Fm) in 2019 (A) and 2020 (B) in different chili pepper cultivars. 0%, control; 50%, deficit irrigation; 100%, optimum WS; HET, Hetényi Parázs; UNIK, Unikal; UNIJ, Unijol; HAB, Habanero.

In second-year outdoor experiments, the difference in chlorophyll fluorescence as associated with the water stress could not be measured as the other researchers found this effect can be explained by the genotype similarity for the examined varieties. Zhou et al. 2017 found both a significant and non-significant reduction in Fv/Fm, depending on the genotypes in tomato.

4.2. Effect of WS on soil moisture

As the 2019 growing season (Fig. 5A) had less precipitation, all cultivars had lower soil moisture content under unirrigated. In HET, the moisture content in 50% and 100% were significantly higher when compared to that of 0% ($p<0.001$). UNIK had a similar trend to that of HET as soil moisture between 50%, and 100% was significantly higher when compared to 0% ($p<0.001$). The same trend was observed in UNIJ ($p<0.001$) and HAB ($p<0.001$). In HET, UNIK, and UNIJ, moisture content at deficit irrigation was slightly higher when compared to 100%, but that was not the case in HAB. Peppers cultivated under the various WS treatments had a significant influence ($p<0.05$) in the second growing season (2020).

The 50% and 100% WS treatments in all cultivars had the highest values when compared to that of 0%, and the soil moisture in 2020 (Fig. 5B) behaved in the same way as it did in 2019. For all cultivars, the highest value is obtained with 100% WS. HET (21.96 ± 4.0), UNIK (23.07 ± 3.75), UNIJ (24.16 ± 3.26) and HAB (24.30 ± 3.87).

According to the statistical analyses, the HET cultivar was solely affected by soil moisture during September, with 100% WS having the maximum soil moisture (20.48 ± 5.46). The other months had no variation in the WS. The lowest value for the soil moisture content for UNIK and UNIJ was determined in July, August, September, and October with 0% WS.

In case of HAB no significant difference was noticed in July, August, September, and October. With 0% and 100% WS soil moisture value was 11.71 ± 4.28 and 20.68 ± 2.30 respectively.

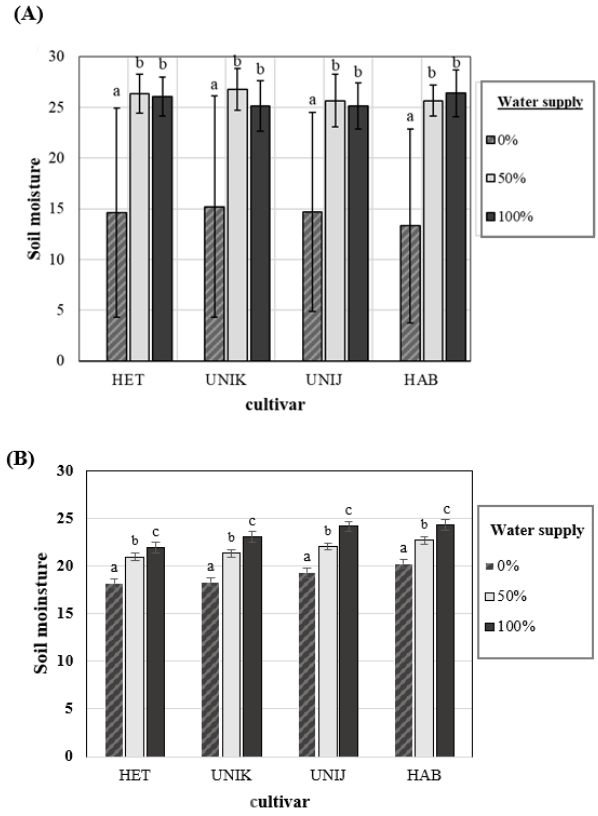

Figure 5: Effect of seasonal WS on soil moisture in 2019 **(A)** and 2020 **(B)** with different chili cultivars

Soil moisture has a significant effect on transpiration and indirectly on the temperature of the leaf surface (Takács et al. 2021; Takács et al. 2019). The threshold value of available groundwater for an appreciable reduction in ETc is approximately 55% in pepper crop (Fernández et al. 2005). Therefore, we collected the soil moisture-canopy temperature data pairs below 20 v/v% water content and performed a regression calculation. We used soil moisture as the independent variable and canopy temperature as the dependent variable. The closest correlation was achieved by fitting it with a power function, whose correlation coefficient (R^2=0.4289; P<0.001) indicates that soil moisture affects leaf temperature by 43% (**Figure 6**). It was the first study to demonstration the correlation between soil moisture and leaf temperature of chili peppers.

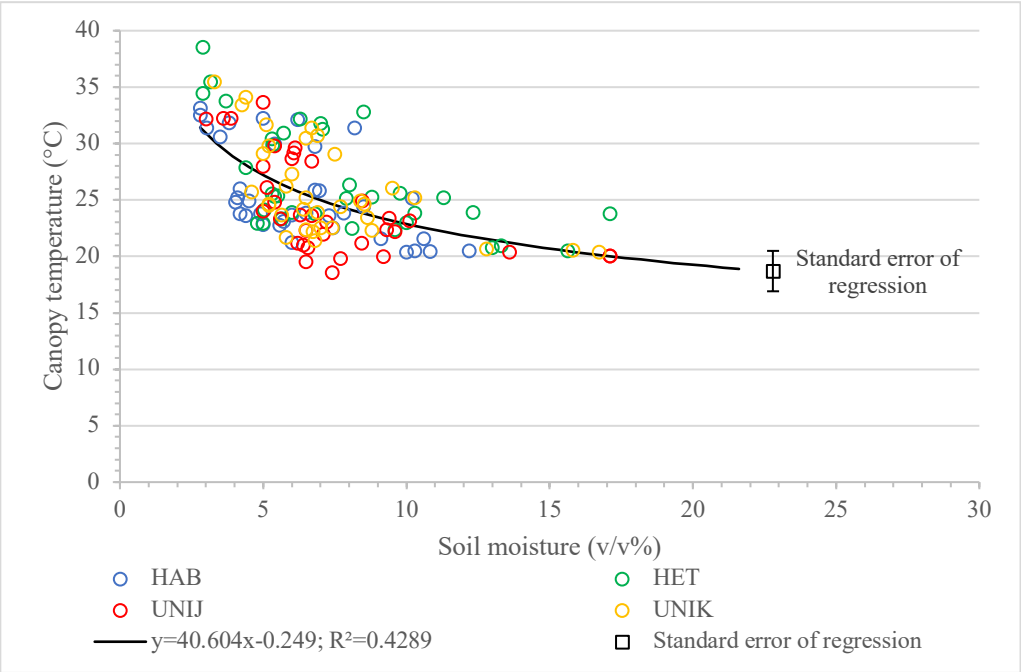

Figure 6: Correlation between the soil moisture and the canopy temperature. Vertical bar represents the standard error of regression (n=144).

4.3. Effect of WS on yield

The effect of WS treatments on the total yield differed from year to year depending on the amount of precipitation and air temperature. For HET cultivar, the highest yield was recorded with 0% WS in 2019, but the highest yield was found with 100% WS in 2020, in which the total yield was significantly lower than that obtained in 2019 (Table3). An opposite tendency was shown by UNIK, which produced higher yield in 2020 than in 2019 with the highest being with 50% WS in 2019 and 100% WS in 2020.

As regards the effect of harvesting time on the yield, it was clear that the amount of precipitation and the temperature 3 weeks before harvest had the most interesting impact. Therefore, the yield fluctuated during the harvest periods (see Table 3 and Appendix 2). The seasonal variation impacted, to a high extent, the yield of the highly pungent UNIJ cultivar. The yield in 2020 was much higher than in 2019 ($P<0.05$-$P<0.001$). The yield increasing WST was 50% in 2019 and 100% in 2020. In case of HAB cultivar, the seasonal variation of the abiotic factors in 2020 caused significant increase in the total yield particularly with a WST of 100%, which increased the yield from 4.4 to 15 t/ha in 2019.

It was also observed (Table 3 and Appendix 3) that the climate of before harvesting time influenced the yield of the cultivars. According to the obtained results, most of the cultivar had the lowest yield at the 3rd harvest. This is because the average temperature in October varied from 10 to 13 °C. This temperature is very low for crop originated from tropical area. The ideal temperature for chili peppers is between 25 and 30 degrees Celsius. The plants suffer from the temperatures between 15 and 32 degrees Celsius. Low nighttime temperatures of less than 14 °C have an impact on plant development, which has an impact on the generation of secondary metabolism and puts the plant under stress at the high temperature.

Table 3*:* The average yield of the peppers cultivated in 2019 and 2020 (n = 4; mean ± SD) based on fresh weight (t/ha)

Cultivar	WST	Total yield (t/ha) 2019	Total yield (t/ha) 2020
HET	0%	24.67±3.34b	15.51±2.03a
	50%	15.96±1.33a	15.81±1.04a
	100%	20.02±3.84b	18.61±0.90b
UNIK	0%	10.83±1.91a	19.20±0.61a
	50%	17.4±2.85b	20.55±0.78ab
	100%	13.24±1.44a	22.51±0.84b
UNIJ	0%	3.76±0.77a	22.94±2.89a
	50%	20.45±3.89c	25.98±2.84b
	100%	9.62±2.37b	33.58±1.55c
HAB	0%	4.41±0.6a	9.03±3.09a
	50%	3.36±0.47a	9.08±3.02a
	100%	15.04±1.56b	9.99±2.94a

Letters represent the difference among WST of cultivar according to Tukey's post hoc test.

Additionally, the raised temperatures might have an indirect or direct impact on agricultural productivity yield the plant growth, morphological features, fruit quality, diameter, and weight, and content of phytochemicals (Gunawardena and De Silva, 2014). As seen from our result the yield at the 1st and 2nd was higher than the at the 3rd harvest because of higher temperature.

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.

4.4. Changes in phytochemicals as a function of harvest and WST

4.4.1. Changes in capsaicinoids

All the cultivars studied had 10 capsaicinoid compounds in their raw materials and dried products, with capsaicin, dihydrocapsaicin, and nor-dihydrocapsaicin being the most abundant. Minor individuals such as the polar (low molecular weight) nor-nor-structured and the less polar (high molecular weight) homo structured capsaicinoids were detected with different amounts in the HPLC profiles depending on the state of ripeness and method of processing **(Figure 7)**.

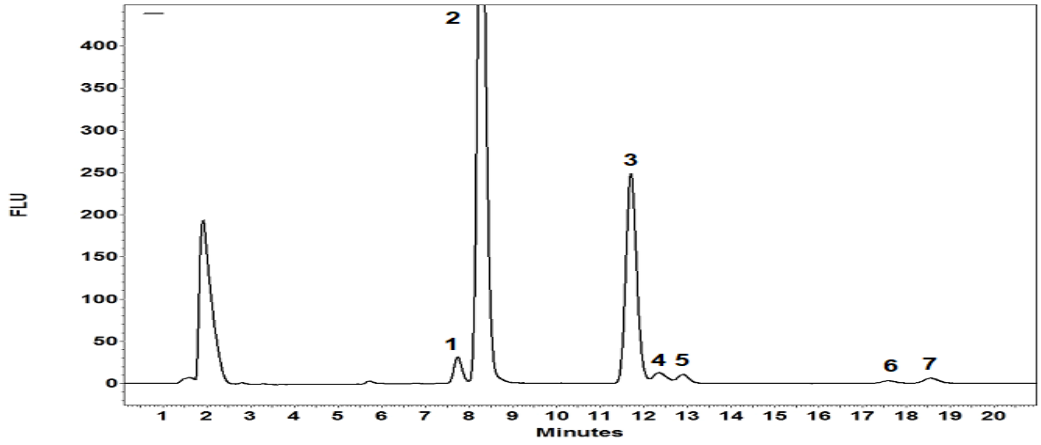

Figure 7: HPLC profile of chili capsaicinoids separated on Purospher Star, 3u, 150 x 4.6 mm column with 52:48 acetonitrile-water. The compounds were detected by FL detector at EX:280 and Em: 290 nm. Peak identification: 1: NDC; 2: CAP; 3: DC; 4: iDC; 5: HCAP; 6: HDCs.

In the evaluation of the obtained results, we focused on the major capsaicinoids since the change in the content of the minor ones was slight and of less interest (Appendix 4-5). It was found that the content of each minor capsaicinoid compounds altered depending on the seasons and the WSTs but without clear and understandable tendencies.

In 2019, with 0% WS there was an increase in the content of NDC from the 1st to the 4th harvest (from warm to cool weather) in HET, UNIK, and UNIJ (Table 4). Such a tendency was not observed with 50% and 100% WS. In case of HAB, the concentration of NDC increased with the proceeding of harvest from the 1st to the 4th irrespectively of the amounts of water supplied to the plants with the highest content was recorded at the 4th harvest ($P<0.01$). It was evident that increase of WS from

0% to 100% resulted in a significant decrease in the content of NDC in 2019 in all cultivars examined except UNIJ, in which the decrease was only at the 4th harvest.

Table 4: Effect of WS on capsaicinoid concentration in the various pepper cultivars for the 2019 growing season. The means are expressed in µg/g fresh weight base ± S.D (n = 4).

Capsai-cinoids	WST/ cultivar	1st harvest	2nd harvest	3rd harvest	4th harvest
NDC					
	HET				
	0%	56.5±4.7Ab	42.9±10.5Ab	63.0±14.8ABb	86.6±15.9Bb
	50%	36.2±1.6ABa	27.1±6.4Aa	41.1±9.3Ba	26.9±4.18Aa
	100%	37.8±3.2Ba	20.3±4.2Aa	23.1±7.1Aa	47.2±8.41Ba
	UNIK				
	0%	46.2±7.0BCa	18.2±2.1Aa	19.7±5.2ABa	64.5±24.9Cb
	50%	32.5±8.3Ba	17.8±2.9Aa	16.1±7.4Aa	19.7±5.6ABa
	100%	42.5±7.7Ba	13.8±2.25Aa	15.7±9.6Aa	30.6±5.5Ba
	UNIJ				
	0%	210.0±40.9ABa	150.7±21.9b	228.2±17.5Bb	350.0±56.3Cb
	50%	269.5±37.4Ba	117.5±7.9Aa	137.5±42.9Aa	239.5±29.3Ba
	100%	241.5±46.6Ba	78.3±14.6Aa	98.0±18.3Aa	284.5±33.5Bab
	HAB				
	0%	108.5±19.6Ab	146.1±8.3Bb	187.2±15.8Cb	
	50%	63.87±8.3Aa	67.3±10.4Aa	105.8±15.7Ba	
	100%	42.87±3.3Aa	61.25±10.5Ba	105.0±0.0Ca	
CAP					
	HET				
	0%	584.1±19.1Cb	375.7±109.78ABa	298.2±65.7Aa	309.6±100.3BAa
	50%	458.6±22.5Ba	300.6±53.8Aa	312.7±77.6Aa	296.3±70.7Aa
	100%	514.8±95.0Bab	238.7±27.7Aa	258.3±72.9Aa	252.0±78.2Aa
	UNIK				
	0%	236.4±42.4Cb	99.4±18.3ABa	68.6±21.4Aa	147.8±55.7Ba
	50%	145.6±28.5Aa	131.6±17.3Aa	104.3±57.5Aa	113.9±34.9Aa
	100%	187.4±38.8Bab	98.2±16.5Aa	91.3±22.5Aa	105.8±13.7Aa
	UNIJ				
	0%	1763.5±46.5Aa	1526.5±56.8Bb	1936.3±216.5Ba	2743.1±429.8Ca
	50%	1992.5±109.4Aa	1900.8±207.4Aa	2099.3±404.3Ba	2507.0±354.2Ca
	100%	1902.8±172.7Aa	1514.3±139.1Aa	2014.2±301.5Aa	2217.2±231.1Aa
	HAB				
	0%	2744.3±317.0Bab	2549.7±181.0ABa	2315.2±171.4Aab	
	50%	2969.7±162.9Ab	2495.5±218.4Aa	2943.5±411.2Ab	
	100%	2392.2±262.6Aa	2381.7±153.0Aa	2202.3±441.9Aa	

DC				
	HET			
	0%	329.7±18.5ABb	236.2±68.5Ab	260.0±73.2ABa	376.7±75.2Bb
	50%	230.1±10.3Aa	163.1±33.2Aab	207.7±56.8Aa	158.9±37.8Aa
	100%	260.0±38.5Ba	123.9±19.9Aa	142.8±47.0Aa	280.1±41.5Bb
	UNIK			
	0%	169.9±36.9Ab	232.4±311.3Aa	64.5±19.8Aa	164.3±63.5Aa
	50%	106.7±21.7Aa	80.1±9.3Aa	79.9±48.1Aa	89.9±28.8Aa
	100%	135.4±18.7Bab	58.6±8.9Aa	61.6±21.3Aa	88.0±9.4Aa
	UNIJ			
	0%	798.8±24.5Ba	1091.6±146.1Ab	1418.3±126.6Aa	2131.3±244.7Bb
	50%	1161.7±223.1Ca	1071.3±113.4Aa	1313.3±422.6Ba	1463.8±453.9Ba
	100%	1046.2±125.5Ca	821.3±160.7Aa	928.3±104.0Ba	1066.6±126.4Ba
	HAB			
	0%	1323.8±155.8Bc	1344.8±56.2Bb	1080.6±76.1Ab	
	50%	1034.25±99.4Ab	979.1±47.0Aa	1036.8±117.3Aab	
	100%	742.8±75.9Aa	886.37±74.1Aa	858.3±111.6Aa	

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test.

In 2020. due to the seasonal variation in the abiotic factors mainly ppt and air temperature, there was substantial variation in the response of NDC to harvest periods (Table 5 and Appendix 5). The content increasing tendency with proceeding of harvest was observed in UNIJ only, in which the concentration of NDC changed from 41-48 µg. g^{-1} at the 1^{St} harvest to 132.178 µg. g^{-1} at the 3^{rd} (last) harvest. NDC in the different cultivars showed different response to the WSTs. The increase in WS impacted in different ways the content of NDC depending on the weather condition before each harvest. However, in most cultivars, with 100% WS there was a slight decrease in the content particularly at the 2^{nd} and 3^{rd} harvest.

The content of CAP tended to decrease with proceeding of harvest toward cool weather in 2019. An opposite response was noted for UNIJ, in which there was an increase in CAP level particularly at the last harvests. It is interesting that content CAP in HAB cultivar did not change significantly as a function of harvesting times.

Under 0%WS conditions, DC concentrations in HET were observed to be significantly ($p = 0.019$) higher when compared to 50% and 100% WS. A similar observation of DC concentration was found in UNIJ ($p < 0.001$). Water supply had no influence on DC concentration in UNIK, although higher amounts were found in unirrigated control. In HAB, as the water supply increased, DC concentration significantly ($p < 0.001$) and progressively decreased.

Since substantial change happened in the abiotic factors in 2020, the response of capsaicinoids to harvesting time was expected to alter. The results showed opposite response to that observed in 2019 towards harvesting time and WS. During harvest periods, in HET and UNIK a significant increase in NDC content took place at only the 2nd harvest and with 0% and 50% WS. The progressive increase in NDC content at all harvest times and with all WSTs happened in the hybrid UNIJ. In case of HAB, the response to harvesting times was like that in HET and UNIK with variation in the highest level being with 100% WS at the 1st harvest.

A slightly significant increase ($P<0.05$) in CAP content at the last harvest under cool weather in HET, UNIK, and HAB, while a decrease in its content was recorded for UNIJ at the 2nd and 3rd harvest as compared to that at the 1st one. These results confirmed that the response of the major pungency compound can be modified by the air temperature and ppt before each harvest. As regards the effect of WSTs on CAP content it can be said that a slight change took place as a function of WS at all harvests with the highest levels being with 0% irrigation.

Table 5: Effect of water supply on capsaicinoid concentration in the various pepper cultivars for the 2020 growing season. The means are expressed in µg/g fresh base weight ± S.D (n = 4)

Capsaicinoid	WST/cultivar	1st harvest	2nd harvest	3rd harvest
NDC				
	HET			
	0%	33.05 ±2.48 Aa	32.49±1.83 Aa	39.14±2.33 Bb
	50%	38.43 ±12.22 ABa	44.48±3.23 Bb	24.39±0.96 Aa
	100%	27.76 ±6.17 Aa	28.87±2.74 Aa	28.70±4.10 Aa
	UNIK			
	0%	17.33 ±6.45 Aa	32.86±0.39 Ba	21.39±4.08 Aa
	50%	13.00 ±3.55 Aa	21.58±7.45 Aa	25.30±2.60 Aa
	100%	18.69 ±4.40 Aa	22.95±6.65 Aa	18.73±4.06 Aa
	UNIJ			
	0%	48.06 ±6.00 Aa	116.26±7.07 Ba	176.14±12.93 Cb
	50%	43.49 ±2.36 Aa	136.95±44.71 Ba	150.53±5.61 Bab
	100%	41.82 ±6.65 Aa	97.60±13.75 Ba	132.43±25.02 Ba
	HAB			
	0%	57.21 ±6.39 Aa	64.59±10.85 Aab	54.61±4.27 Aab
	50%	68.14 ±10.58 Aab	77.36±2.91 Ab	69.86±9.42 Ab
	100%	79.44 ±7.79 Bb	52.18±4.53 Aa	49.33±4.05 Aa
CAP				
	HET			
	0%	274.96 ±9.78 Aa	367.20±32.33 Bab	329.27±50.30 Aa
	50%	356.94 ±46.47 AaB	355.26±61.27 AaB	259.45±26.88 Aa
	100%	299.97 ±27.16 Aab	332.53±1.48 Aa	324.07±10.92 Aa

	UNIK			
	0%	133.44 ±33.20 Aa	260.24±22.78 Bb	212.32±32.64 Bb
	50%	108.86 ±15.02 Aa	150.95±36.03 ABa	173.85±2.20 Bab
	100%	108.66 ±9.51 Aa	135.15±9.54 Aa	136.05±21.08 Aa
	UNIJ			
	0%	2841.54 ±156.40 Ba	2384.00±44.76 Ab	2206.16±118.49 Ab
	50%	2809.57 ±162.05 Ba	2179.55±66.32 Aa	1924.39±47.53 Aab
	100%	2875.77 ±411.00 Ba	2176.51±88.65 Aa	1756.51±185.74 Aa
	HAB			
	0%	1736.00 ±43.99 Aa	2695.02±10.43 Ba	2235.48±360.48 ABa
	50%	2032.15 ±185.59 Aa	3257.22±272.51 Ba	2845.36±168.22 Ba
	100%	1920.51 ±163.04 Aa	2918.60±318.12 Ba	2541.83±145.69 Ba
DC				
	HET			
	0%	188.27 ±2.65 Aa	274.99±42.67 Bb	217.75±21.75 ABb
	50%	244.96 ±50.07 Ba	217.58±8.39 ABab	159.55±22.52 Ba
	100%	186.05 ±32.67 Aa	185.27±5.05 Aa	172.39±11.71 Aab
	UNIK			
	0%	96.40 ±26.65 Aa	179.66±15.18 Bb	119.55±5.43 Aa
	50%	74.49 ±11.43 Aa	93.84±9.12 Aa	126.42±5.48 Ba
	100%	82.90 ±7.35 Aa	95.87±3.86 Aa	98.22±21.38 Aa
	UNIJ			
	0%	947.93 ±51.57 Aa	1204.45±48.79 Ba	1341.31±135.23 Bb
	50%	863.81 ±43.67 Aa	1168.09±263.72 Aa	1178.30±51.24 Aab
	100%	887.61 ±91.79 Aa	938.72±73.27 Aa	1014.35±153.05 Aa
	HAB			
	0%	925.08 ±234.25 Aa	1099.86±38.21 Aab	1242.30±122.27 Ab
	50%	883.96 ±85.66 Aa	1162.26±39.70 Bb	918.36±118.12 Aa
	100%	895.45 ±41.25 Aa	991.56±79.19 Aa	860.03±35.00 Aa

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test.

The response of (DC) was different from that of CAP. The highest concentration was determined at the 2nd harvest in all the cultivars with o% irrigation. With increase of WS there was either slight decrease or no significant change as a function of proceeding of harvest towards the last one. This indicates that water deficiency and warm weather before harvest is favorable for dehydrogenation of CAP to DC, WS had no effect on DC concentration under no irrigation conditions for all cultivars examined. Nevertheless, concentration was found to be significantly lower in 50% and 100% of the second ($p \leq 0.001$) and fourth ($p=0.002$) harvests. As the WS increased, DC concentration in HAB decreased significantly ($p \leq 0.001$) in the 1st harvest. Also, in the 2nd harvest, concentration was found to be significantly ($p \leq 0.001$) lower in 50% and 100% when compared to 0%. In the fourth harvest, concentration was found to be significantly ($p=0.032$) lower in 50% and 100% when compared to 0% even though, no change was detected between 50% and 0%.

The biologically active compounds vary in quantity according to the genotype, ripening, and cultivation system (Batiha et al. 2020). These metabolites are a natural defense against biotic and abiotic mechanisms and can be synthesized in quantity by the plant during stress conditions (Wahyuni et al. 2013). In general, for all crop production, the water is the most limiting factor in production. The effect of the WS in our study, did not have significant differences between 0%, 50% and 100% and this data may have been due to the rainfall rates that were higher for these months, with this the factor WS was not significant. The author (Sung et al. 2005) studied the water stress in three different varieties and in two of them "Home flavor" and "Hungariana" the effect of WS on the capsaicin content in these two varieties was less and this could be because of the pepper cultivar, and according to the author different cultivars have different internal metabolic system being differently affected by external abiotic, biotic, and physical factors. Another factor that also decreases the capsaicin is a fruit age, and this effect is because the capsaicin synthase activity was significantly reduced in the placenta of the fruits of this age and under water stress and this effect can be associated with the action of the peroxidase activity (Ruiz-Lau et al. 2011). In addition to water stress, other abiotic factors such as heat stress can also influence the production of capsaicin in plants, During the months of the harvest, Hungary was under high temperature than usual for August and September, and under lower temperature in October. This temperature also could contribute to the content of capsaicin. Chili pepper is a plant originally from tropical regions and the optimum temperature is between 25 °C and 30 °C. Temperatures below 15 °C and excess 32 °C can affect the plant in general. Low night temperatures under 14 °C affect the development of the plant, consequently affecting the production of secondary metabolism, plant under stress because of high temperature and drought stress, create a differential synthesis of carotenoid in the internal metabolic of the plant (Pressman et all 1998). Thanopoulos et al. (2013) worked with chili pepper in Mediterranean region where the chili was grown under high temperature and low temperature. The authors stated that that the cultivation in autumn yielded bigger fruits, but their nutritional value was lower.

4.4.2. Changes in Carotenoids

The HPLC technique used to separate the various carotenoids yielded excellent separation of roughly 56 carotenoids in the form of free, monoesters (ME), and diesters (DE) bearing distinct fatty acid moieties (Figure 8). Among 56 detected carotenoids 41 have been identified earlier both in fresh and dry chili peppers as previously reported (Duah et al. 2021). Unsaturated fatty acids have been found in at least four carotenoid diesters. Saturated and unsaturated aliphatic chains with 12 and 18 carbon atoms made up the fatty acid moieties. The fatty acid moiety of carotenoids is

expected to vary, resulting in changes in their stability throughout processing and storage of chili pepper products (Appendix 1).

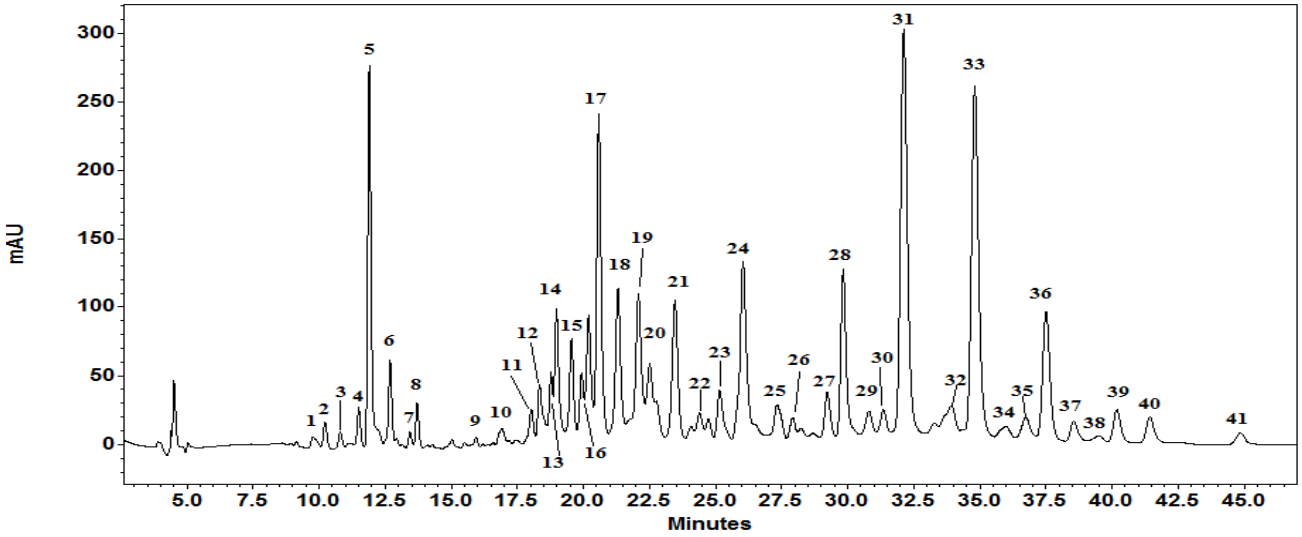

Figure 8: HPLC profile of chili pepper carotenoids separated on C18, 3u, 240 x 0.46 mm column with gradient elution of (B) Acetonitrile-isopropanol-methanol in (A) methanol-water and DAD detection at 460nm. Identification of peaks as in Appendix 1

To achieve a better understanding and to have a meaningful discussion of the results the individual carotenoids analyzed by HPLC were arranged in different groups that show the changes in the quality and nutritional value of the examined chili peppers.

The total carotenoid concentration was determined by the sum of concentration of the individual carotenoids identified on the HPLC chromatogram. The effect of water supply on the total carotenoid groups in the chili cultivars cultivated in 2019 was assessed (Figure 9), whereas the changes in the content of all groups caused by WSTs and cultivars are shown in Appendix 6.

A lower concentration of carotenoid compounds was found in the 1st harvest when compared to the 2nd, 3rd, and 4th harvests. Between the cultivars, HET and UNIJ recorded higher concentrations of carotenoid compounds when compared to UNIK and HAB. The concentration of HAB was the lowest among all the pepper cultivars. Between the WS treatments in the 1st harvest, no influence was found in HET, even though a slight decline in concentration was recorded as the WS increased. A similar trend was recorded in UNIJ peppers. However, in UNIK, a significantly ($p=0.050$) lower carotenoid concentration was recorded in 100% when compared to 0%. It was found in HET that total carotenoids concentration decreased significantly ($p=0.038$) in 50% when compared to 0%.

However, in UNIK, UNIJ, and HAB, no significant change in concentration was recorded even though a slight decline in concentration as WS increased was found in UNIJ. As concerns the total carotenoid concentration at the 3rd harvest, it decreased as the WS increased in all cultivars. Nonetheless, in HET and HAB, no significant differences were found in them.

An increase in water supply significantly ($p = 0.007$) lowered free caps concentration in HET even though between 50% and 100%, no change in concentration was recorded. It was observed in UNIK and UNIJ cultivars that water supply had no influence on free caps concentration. HAB, on the other hand, had a significant ($p < 0.001$) decrease in free caps concentration as water supply increased, and under 100% WS conditions, no free capsanthin was detected. The amount pf Caps ME in HET was found to be significantly ($p = 0.021$) lower under 50% when compared to 0% irrigation. In addition, in HAB, caps ME concentration were significantly ($p = 0.041$) lower under DI when compared to 0% and 50%. As the water supply increased, caps ME concentration in UNIK significantly ($p = 0.007$) increased in 50% and 100% when compared to 0% WS. In UNIJ, water supply treatments did not influence caps ME concentration. Considering caps DE concentration in HET, under 0% conditions, a significantly ($p = 0.029$) higher amount was recorded when compared to 50%. A similar trend was recorded in UNIJ; caps DE concentration was significantly ($p < 0.001$) higher with 0% when compared to 50%. WS. In UNIK, water supply did not influence caps DE amount. Caps DE amount in HAB increased significantly ($p = 0.006$) under 50% and 100%WS conditions when compared to 0% WS.

Under 100% WS conditions, concentration in UNIK significantly ($p=0.013$) decreased when compared to 0%. Also, in UNIJ, a significantly ($p=0.036$) lower concentration was recorded in 100% when compared to 0%. In the fourth harvest, higher concentrations were found in HET and HAB under 100% even though there were no significant differences between treatments. Likewise, in UNIK and UNIJ, there were no significant differences between WS treatments, even though a slight decline in concentration was evident as the WS increased.

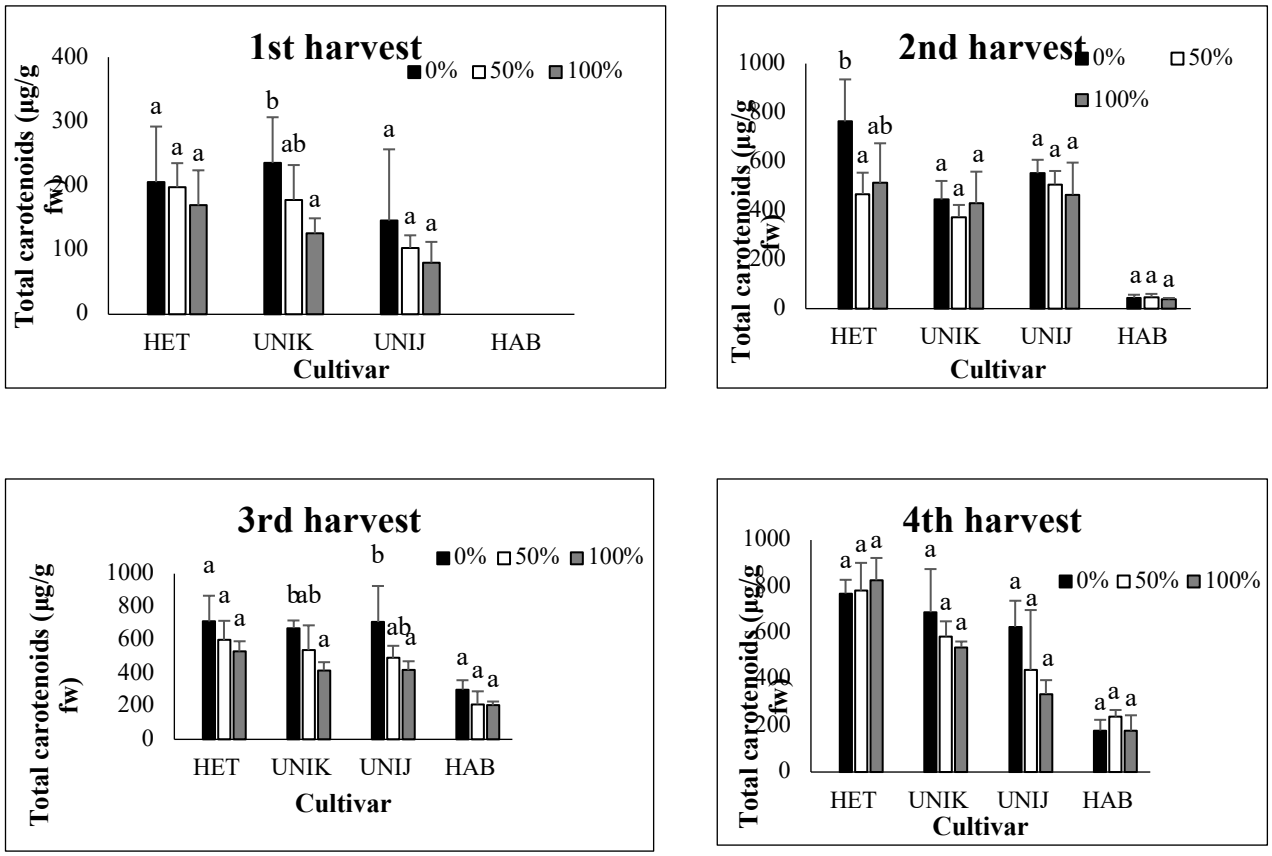

Figure 9: Mean concentration of total carotenoids present in the chili pepper cultivars at the various harvesting stages in the 2019 growing season.

The carotenoid groups and their response to WSTs and harvest times in the 2020 cultivation year are shown in Table 6 and Appendix 7. The increment of WS from 0% to 50% and 100% resulted in a reduced content of the total free red xanthophylls (FRX) in HET at the 1st and 2nd harvests, but at the last harvest when the climate variables especially the temperature and precipitation, were different, it did not significantly affect the concentration of this group. The cultivar UNIK, unlike HET, responded inversely to the change in WS and harvesting times. The content-decreasing effect of increased water stress was noticed only at the 3rd harvest, while at the 1st and 2nd harvest it caused the level of FRX to increase. In the case of the highly pungent hybrid UNIJ, the WSTs resulted in a remarkable decrease in the level of FRX at all harvest times. In the non-spice peppers cultivar HAB, the positive (increasing) effect of water stress was only noticeable at the 1st harvest, but it dropped at the 3rd harvest.

Table 6: Effect of harvesting time and WS treatments on carotenoid concentration in the 2020 season. The means are expressed in µg/g fresh weight base ± S.D (n = 4)

Carotenoid	WST/ Cultivar	1st harvest	2nd harvest	3rd harvest
Total Red				
	HET			
	0%	421.30 ± 13.94 Ba	568.93 ±29.00 Cb	267.73±20.50 Aa
	50%	407.26 ± 6.32 Ba	352.68 ±44.79 Ba	236.41±15.70 Aa
	100%	433.82 ± 26.10 Ba	386.39 ±28.20 Ba	241.05±11.72 Aa
	UNIK			
	0%	488.58 ± 42.86 Bb	386.04 ±22.10 Bb	212.49±79.37 Aa
	50%	320.35 ± 30.16 Ba	313.57 ±24.74 Ba	188.56 ± 3.73 Aa
	100%	391.67±42.48 Bab	347.85 ±22.39 Bab	155.23±20.43 Aa
	UNIJ			
	0%	340.56 ±11.52 Cb	278.75 ±23.78 Ba	211.66±16.46 Aa
	50%	335.85 ± 32.85 Bb	291.07±69.59 ABa	200.17±20.50 Aa
	100%	272.46 ± 6.37 ABa	322.87 ±12.35 Ba	226.03±37.72 Aa
	HAB			
	0%	95.12 ± 10.56 Aa	162.51 ±22.65 Ca	102.46±11.40 Ba
	50%	151.15 ± 8.35 Ab	169.43±16.03 Bab	108.92±10.68 Ba
	100%	159.15 ± 19.26 Ab	238.42 ±42.40 Bb	166.44±15.92Ab
Total Yellow				
	HET			
	0%	255.98 ± 8.26 Bb	314.67 ±24.70 Cb	156.53±13.32 Aa
	50%	205.27 ± 15.87 Ba	203.07 ±20.34 Ba	144.01 ± 4.60 Aa
	100%	177.09±21.6 ABa	215.55 ±21.72 Ba	155.14±13.81 Aa
	UNIK			
	0%	234.11±16.59Ba	235.59 ±15.15 Bb	166.72±22.14 Ab
	50%	213.13 ± 18.50 Ba	188.55 ±5.69 Ba	139.82 ± 6.08 Aab
	100%	222.61 ± 16.46 Ba	205.13 ±13.29 Ba	100.27 ± 15.64 Aa
	UNIJ			
	0%	218.07 ± 10.60 Aa	224.33 ±11.35 Aa	225.21 ± 6.47 Ac
	50%	180.69 ± 30.67 Aa	254.34 ±38.79 Ba	158.57 ± 10.22 Aa
	100%	174.49 ± 8.29 Aa	226.09 ±7.45 Ba	186.77 ± 5.93 Aa
	HAB			
	0%	68.54 ± 5.23 Aa	137.79 ±15.27 Ca	103.22 ± 17.22 Ba
	50%	103.81 ± 7.00 Ab	148.49 ±12.03 Ba	122.03 ± 18.91 Aa
	100%	103.14 ± 9.23 Ab	161.09 ±4.17 Ba	177.12 ± 5.15 Bb
R/Y				
	HET			
	0%	1.65 ± 0.07 Aa	1.81 ±0.05 Ba	1.71 ± 0.03 ABb
	50%	1.99 ± 0.12 Bb	1.73 ±0.05 Aa	1.64 ± 0.07 Aab
	100%	2.46 ± 0.15 Bc	1.80 ±0.05 Aa	1.56 ± 0.06 Aa
	UNIK			
	0%	2.09 ± 0.05 Bc	1.64 ±0.08 ABa	1.25 ± 0.36 Aa
	50%	1.50 ± 0.04 Ba	1.66 ±0.09 Ca	1.35 ± 0.04 Aa
	100%	1.76 ± 0.07 Bb	1.70 ±0.06 ABa	1.55 ± 0.04 Aa

	UNIJ			
	0%	1.56 ± 0.07 Ca	1.24 ±0.06 Bab	0.94 ± 0.10 Aa
	50%	1.88 ± 0.21 Ba	1.14 ±0.14 Aa	1.26 ± 0.14 Aa
	100%	1.56 ± 0.04 Ba	1.43 ±0.01 ABb	1.21 ± 0.17 Aa
	HAB			
	0%	1.39 ± 0.06 Ca	1.18 ±0.04 Ba	1.00 ± 0.09 Aa
	50%	1.46 ± 0.02 Ca	1.14 ±0.08 Ba	0.90 ± 0.09 Aa
	100%	1.54 ± 0.16 Ba	1.48 ±0.22 Ba	0.94 ± 0.12 Aa
β-carotene				
	HET			
	0%	33.62 ± 1.71 Aa	65.38 ±4.12 Bb	68.34 ± 5.37 Ba
	50%	45.65 ± 6.60 Aa	50.25 ±2.96 ABa	59.54 ± 1.70 Ba
	100%	44.69 ± 5.23 Aa	49.67 ±4.35 Aa	63.49 ± 6.13 Ba
	UNIK			
	0%	51.22 ± 2.23 Ab	64.52 ±2.13 Bc	57.64 ± 8.48 ABb
	50%	39.93 ± 7.37 Aa	45.86 ±3.23 Aa	43.65 ± 4.04 Aab
	100%	35.15 ± 0.71 Aa	57.92 ±1.81 Bb	39.21 ± 6.59 Aa
	UNIJ			
	0%	47.59 ± 2.94 Aa	75.83 ±7.27 Ba	67.86 ± 5.93 Bb
	50%	35.91 ± 8.83 Aa	72.52 ±12.68 Ba	47.57 ± 5.18 Aa
	100%	36.11 ± 4.02 Aa	64.47 ±4.99 Ba	55.27 ± 2.77 Ba
	HAB			
	0%	19.74 ± 1.19 Aa	67.34 ±3.82 Ba	53.17 ± 10.14 Ba
	50%	32.91 ± 4.13 Ab	67.08 ±5.44 Ba	63.03 ± 13.67 Ba
	100%	32.96 ± 3.88 Ab	69.71 ±0.81 Ba	104.71 ± 5.26 Cb
Total vitamin A precursor				
	HET			
	0%	45.97 ± 3.38 Aa	84.87 ±5.81 Bb	77.10 ± 5.92 Ba
	50%	56.16 ± 7.15 Aab	61.37 ±3.48 ABa	70.10 ± 1.64 Ba
	100%	65.59 ± 5.77 Ab	67.97 ±6.01 Aa	71.17 ± 7.13 Aa
	UNIK			
	0%	74.98 ± 2.40 Ab	80.78 ±4.31 Ac	69.79 ± 9.87 Ab
	50%	61.42 ± 8.05 Aa	54.12 ±3.23 Aa	52.18 ± 4.11 Aab
	100%	55.95 ± 1.81 Aa	71.63 ±1.30 Bb	45.62 ± 7.58 Aa
	UNIJ			
	0%	66.99 ± 4.39 Aa	98.32 ±8.66 Ba	91.47 ± 7.39 Bb
	50%	50.11 ± 10.65 Aa	102.77 ±21.80 Ba	56.62 ± 2.46 Aa
	100%	51.57 ± 2.96 Aa	88.26 ±7.26 Ca	68.87 ± 3.73 Ba
	HAB			
	0%	26.36 ± 1.37 Aa	75.71 ±5.26 Ba	66.32 ± 10.78 Ba
	50%	42.82 ± 5.87 Ab	78.34 ±6.09 Ba	77.87 ± 13.50 Ba
	100%	44.37 ± 3.56 Ab	85.79 ±0.82 Ba	126.70 ± 7.07 Cb

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test.

The monoesters of red xanthophylls (ME-RX) in HET, UNIK, and HAB showed response to WSTs like that of FRX. However, there was an alteration in the tendency of change in UNIK and UNIJ as

a function of increase in WS at the 2nd harvest. The increase of WS to 100% caused the level of ME-RX to significantly increase.

As for the diesters of red xanthophylls (DE-RX), the positive effect of WS was evident in HET at the 1st harvest, and in HAB at all harvests. The inverse influence was noticed in UNIK and UNIJ where the increase in WS had a negative impact on the content of DE_RX, and furthermore none of the examined cultivars exhibited significant change at the last harvest.

The yellow colored free xanthophylls (FYX) behaved by similar way to that exhibited by the (FRX) towards the changes in WS in HET but varied from that in UNIK at the 2nd harvest where no significant changes took place as a function of increased WS. The lowest level of such fraction was recorded with100% at the 4th harvest.

The fraction of monoesters of yellow xanthophylls (ME-YX) in the fruits of HET responded to WS by similar way to that observed with the ME-RX at all harvest except at the 4th one, when no significant effect was found. The content-decreasing impact of WSTs on ME-YX was only evident at the 1st harvest of UNIJ, and a slight increase (not significant) was observed at the next harvests. It is of special interest that in the freshly consumable HAB cultivar ME-YX exhibited significant increase as the WS increased from 0% to 50% and 100%, at all harvests revealing that under water stress biosynthesis of such fatty acid esters is activated most likely via activation of relevant enzymes.

The diester fraction of the major yellow carotenoids (DE-YX) showed somewhat different response to WSTs and harvests. In general, the promotive effect of water stress was noticed in HET at the 3rd harvest, in UNIK at the 1st harvest, in UNIJ at the 2nd harvest and in HAB at the 2nd and 3rd harvests with the highest level being recorded for UNIJ at the last harvest when the temperature was substantially lower (cooler) than at the 1st and 2nfd harvests. Such climate conditions were not favorable for the formation of most of carotenoid groups in other cultivars.

The total red or yellow carotenoids followed the sum of mono and diesters as the major constituents of these groups. The most interesting finding is that in all cultivars examined except HAB the cool weather at the last harvest was not favorable for carotenoid biosynthesis with negative effect of the high-water stress, which showed positive impact in HAB on the total red and yellow carotenoids. The ration of red to yellow carotenoids is an important index of chili pepper quality as it gives information on chemical stability of red or yellow pigment and the real change in the color of chili products. The maximum values of R/Y 2.04 and 2.43were found in HET and UNIK with 100% and 0% WST respectively indicating then variation between the two cultivars in carotenoid response to

water stress. With the proceeding of harvest there was marked decrease in the ratio in all cultivars that confirms the lower biosynthesis rate or chemical stability of red-colored carotenoids as compared to those of yellow ones under cool weather and high-water stress conditions.

From the biological point of view, sum of lutein, zeaxanthin β-cryptoxanthin and β-carotene received special interest in the present work. All these carotenoids are bioactive plying important roles in human and animal bodies. Lutein and zeaxanthin are essential compounds in the function of macular membranes and visuality, while others are highly active antioxidant preventing biochemical oxidation and, thus, participate in the reduction of some cardiological diseases and cancers. The different cultivars varied in the response of the sum of L+Z to water stress and harvest time. In HET, the water stress had negative impact at the 1st and 2nd harvest, while at the last harvest there was remarked decrease in the level of such group even with 0% WS. It is evident that the harvesting time did not alter the response of this group to 50% WS. In UNIK the response of the group was different from that in HET. The highest level was recorded at the 2nd harvest, at which only slight content-decreasing impact of water supplies was observed. The greatest negative effect of WSTs and harvest time was found at the last harvest followed by the 1st harvest indicating that UNIC cultivar needs warm season to synthesize more lutein and zeaxanthin. As for the highly pungent hybrid UNIJ, like in UNIK the highest content was determined at the 2nd harvest and the lowest at 1st harvest. The high-WS significantly affected the values for the sum of L+Z only at the last harvest with 50% being the most content-decreasing as compared to other treatments. In the case of HAB the fruits distributed the lowest quantities of such carotenoid group, which increased significantly at the 2nd harvest. The level improving WS was observed with 100% and at only the 1st and 3rd harvest.

The metabolic pathway of β-cryptoxanthin, the 2nd vitamin A precursor in chili peppers, seemed to prefer the conditions of the 1st harvest in HET and the 2nd harvest in the other cultivars. As other carotenoids did, β-cryptoxanthin decreased at the last harvest in most cultivars except HAB, in which slight changes took place.

The main precursor of vitamin A in the chili peppers is β-carotene, which has special importance as bio-antioxidant. Its biosynthesis showed interesting response to the WSTs and harvesting time. In HET there was increasing tendency from the 1st to the last harvest, while the conditions of the 2nd harvest yielded peppers with the highest levels of β-carotene in UNIK and UNIJ. The water stress with 100% WST had positive effect at the 1st harvest of HET. In UNIK and UNIJ there was a content-decreasing impact of water stress at all harvest times, and the maximum levels were determined at the 2nd harvest. HAB exhibited interesting alteration in the response to water stress

and harvesting time. The positive impact of water stress was at the 1st and 3rd harvest with the maximum concentration being recorded with 100% WST at the last harvest affirming the positive effect of cool days prior to harvest, at the end of the growing season, on the pathway of β-carotene biosynthesis.

The total carotenoid also varied with respect to the cultivar and the harvest time (Figure 10). The 1st and 2nd harvests often gave pepper fruits with the highest total carotenoid levels for most cultivars and treatments. The effect of the water stress on the total carotenoid content seems to be climate-and genotype-dependent. Increasing the WS from 0% to 100% accompanied by a slight or great decrease($p<0.05$) in the total carotenoid concentration in the cultivars with low or medium pungency. In the highly pungent cultivars, the response water stress substantially varied particularly in the freshly consumable HAB. The water stress caused the total carotenoid quantity to significantly increase ($p<0.05$) at all harvest times. Nevertheless, in UNIJ there was a slight but not significant content-increasing effect of water stress at the 2nd harvest only, while at the 1st and 3rd harvest a slight decreasing effect was evident.

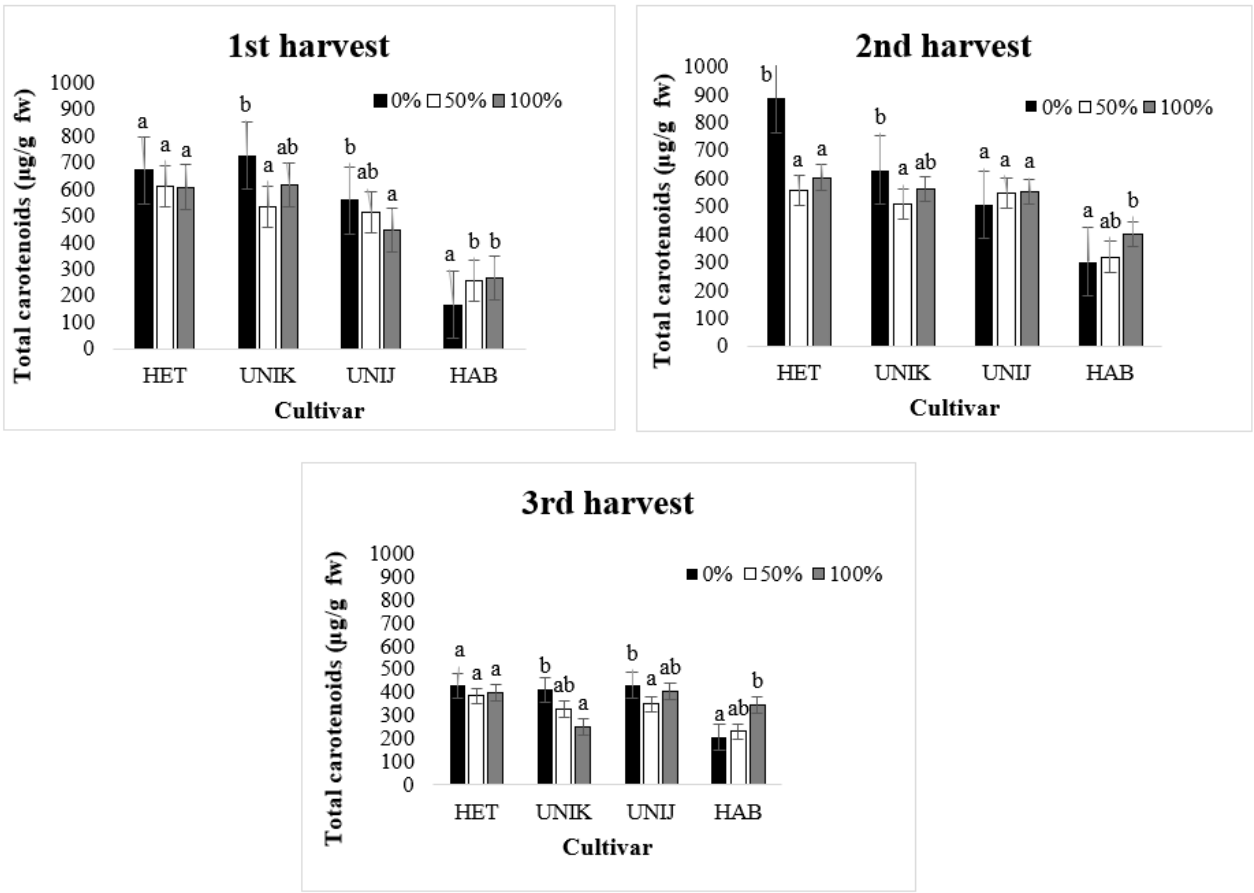

Figure 10: Mean concentration of total carotenoids present in the chili pepper cultivars at the various harvests in the 2020 growing season.

The irrigation frequency is one of the factors that also with the ripening stage can significantly reduce the carotenoid compounds. Marin et al (2009) found that with low irrigation red peppers had the highest carotenoid and provitamin A contents. The deficit of water affects the plant growth, and this consequence affects the physiological and biochemical processes, such as photosynthesis, all metabolism in the plant and may impact on the growth promoters too (Hayat et al., 2008 and Khan et al., 2003). In our research the highest difference happened not in the WS difference but in the harvest period, and in general the total of carotenoid showed higher concentrations in the first and 2nd harvest and lower in the 3rd harvest. Kırnak et al. (2016) reported that effects of irrigation, cultivar, and their interactions on β-carotene content of bell pepper. We also found the same correlation and the effect of WS on carotenoid compounds depends on the harvest period and the cultivar. In our research the harvest period affected more the β-carotene than the WS in some cultivars and probably because of the rainfall during this period could also affect the results for the water treatment. Other authors already reported that the carotenoids compounds are affected by maturity, genotype, (Lee et al., 1995) and harvest time (Reverte et al., 2000). When pepper is at immature stage, the pathway starts from lycopene and synthesizes other carotenoids such as α-carotene, zeaxanthin, and lutein. In contrast, when the pepper is at a mature stage, the β-carotene pathway begins with lycopene and the carotenoids are synthesized in the order, β-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin according to (Ha et al. 2007).

4.4.3. Changes in vitamin C

The HPLC separation of vitamin C from other organic acids is shown in Appendix 11. Peak purity test indicated that L-ascorbic acid did not overlap with other organic acids that made accurate the quantitative and qualitative determination of the vitamin. In both growing seasons, WST and harvest time affected the level of the vitamin. In general, vitamin C tended to increase with preceding of harvest from the 1st to the last. It is to be mentioned that at the 1st harvest the temperature was higher than that at the last harvest, which is usually cool. In 2019 the warm climate continued till the beginning of November giving possibility to get the 4th harvest, while in 2020 the soil frost started at the end of October limiting the number of harvests to 3.

Except in HET, there was a remarkable increase in the content of vitamin C of the different cultivars throughout the harvests. The extent of increase in vitamin C was influenced by genotype and WS.

The ration of vitamin C amounts at the last and 2nd harvest was related to that at the 1st harvest to get the % change took place during harvests (last/1st; 2nd/1st). In 2019 (Table 7), the increase at the 4th harvest ranged between 18% and 138% with no WS, between 0% and 147%, with 50% WS, and between 20% and 148% with 100% WS among the cultivars studied. The highest increase was recorded for HAB cultivar while the lowest was found in HET, in which vitamin C showed no response to the climate of each harvest with 50% WS. In the case of 2nd/1st ratio, the negative response (decrease) of vitamin C content was repeatedly evident in most cultivars except HAB, which exhibited positive response but not as high as happened at the 4th harvest. The lowest %decrease in vitamin C was 12% in HET with 0% WS and the highest was 53% in UNIJ with 50% WS. These results revealed the effect of the interaction between WS and the abiotic factors at certain harvesting time (Table 7).

Table 7: Effect of harvesting periods and WS treatments on vitamin C content in 2019. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Vitamin C 2019				
WST/ Cultivar	**1st harvest**	**2nd harvest**	**3rd harvest**	**4th harvest**
HET				
0%	3725.2±521.4ABa	2925.4±194.6Ab	3387.9±250.8Ab	4397.5±76.9Bb
50%	3656.2±215.6BCa	2521.80±171.9Aab	3175.5±152.8Bb	3683.6±252.1Ca
100%	3337.5±367.6Ba	1887.13±216.8Ab	2487.8±172.1Aa	4021.9±311.7Bab
UNIK				
0%	3545.6±388.4Bb	1926.4±177.6Aa	2653.2±175.7Aa	4184.0±286.2Ca
50%	2874.4±162.6Ba	1731.7±143.7Aa	2587.5±311.5Ba	3753.7±202.8Ca
100%	2737.5±137.4Ca	1664.0±62.7Ba	2595.1±190.5Aa	4006.6±145.6Ba
UNIJ				
0%	2615.9±133.2Bab	1720.3±153.4Ab	2646.6±104.5Bb	3624.4±329.4Ca
50%	3028.8±815.6ABb	1432.3±106.3Aa	2414.4±78.1Bab	3488.6±244.5Ca
100%	1956.8±56.9Ba	1296.0±139.9Aa	2227.2±158.1Ca	3842.3±96.7Da
HAB				
0%	1357.1±81.4Ab	2136.3±200.6Bb	3223.7±118.6Cb	
50%	1183.8±371.4Aa	1531.9±188.0Aa	2919.3±146.7Ba	
100%	1157.1±73.3Aa	1745.4±259.9Bab	2870.0±148.8Ca	

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test.

In 2020 (Table 8), the abiotic factors of the growing season particularly 3 weeks before the last(3rd) harvest promoted to a high extent, the biosynthesis of vitamin C in UNIK and UNIJ. The maximum increase was stated in pepper pods from UJIJ, in which the % increase ranged between 66% and

196% depending on the WSTs. The seasonal variation caused the cultivar HAB to rank 2nd in the % increase of vitamin C as a function of the 4th harvest climate (coolness). As regards the negative change took place at the 2nd harvest, UNIK cultivar showed the greatest decrease in vitamin C (20-36%) followed by HAB (13-19%). In UNIJ only with 50% WS there was a negative effect of climate on the vitamin's level while at 0% and 100% WS the abiotic factors still have positive impact, but not as great as found at the last harvest.

The afore-mentioned results confirmed that the changes in climate variables from year to year can alter the response of chili peppers with respect to biosynthesis of vitamin C. It is important to mention that increasing the WS to approach a water stress state for the plants is not favorable for biosynthesis of vitamin C in all chili cultivars studied with HET being the most stable one towards changes in climate variable.

Table 8: Effect of harvesting time and WS treatments on Vitamin C content in 2020. The means are expressed in µg/g fwb ± S.D (n = 4).

	Vitamin C 2020		
WST/ Cultivar	**1st harvest**	**2nd harvest**	**3rd harvest**
HET			
0%	3847.72 ± 281.79 Aa	3731.88 ± 295.68 Aa	3455.05 ± 185.89 Aa
50%	4005.51 ± 91.34 Aa	3581.24 ± 109.78 Aa	3667.10 ± 285.23 Aa
100%	4081.51 ± 562.86 Aa	3571.25 ± 114.97 Aa	3961.76 ± 568.49 Aa
UNIK			
0%	4341.36 ± 623.93 ABa	3470.23 ± 243.72 Aa	4855.63 ± 365.56 Ba
50%	4756.96 ± 67.43 Ba	3056.53 ± 90.04 Aa	4901.34 ± 110.63 Ba
100%	4683.41 ± 233.10 Ba	3383.09 ± 362.16 Aa	4504.62 ± 402.08 Ba
UNIJ			
0%	1575.73 ± 48.66 Aa	2114.40 ± 156.58 Aa	3921.17 ± 411.59 Ba
50%	2225.90 ± 74.40 Bb	2013.17 ± 32.04 Aa	3715.10 ± 68.12 Ca
100%	1369.88 ± 198.90 Aa	2436.62 ± 110.43 Bb	4049.99 ± 238.80 Ca
HAB			
0%	1843.91 ± 382.40 Aa	1947.58 ± 24.31 Ab	2847.56 ± 143.11 Ba
50%	2043.44 ± 95.52 Aa	1780.23 ± 48.97 Aa	2924.87 ± 515.28 Ba
100%	2111.11 ± 83.10 Aa	1708.91 ± 88.69 Aa	2668.95 ± 348.79 Ba

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test.

Studies have reported that with the lack of irrigation the vitamin C content decreases (Ahmed et al. 2014; Marín et al., 2009). Another factor that is related to vitamin C is the leaf temperature. According to Mahendran and Bandara, (2000) when increases because of the moisture stress the

leaf temperature decreases the concentration of vitamin C in the pods of pepper. In a previous study (Duah et al 2021) on the effect of WS in open field for two years it was found that the low temperature and low precipitation could also contribute to the higher amount of vitamin C depending on the weather and the rainfall period.

In the research on outdoor cultivation of chili pepper (Nurzyńska-Wierdak et al. 2021), it has been found that the irrigation had some impact on the vitamin C level. In non- irrigated plants (134.71 mg per 100g fwb) it was higher than in irrigated ones (114.89 mg per 100 g fwb). The obtained results were not consistent with some literature data. The reason for that might be the rainfall period and the sensitivity of vitamin C to changes in environmental conditions. Vitamin C gets oxidized very rapidly when exposed to high temperatures (Davies et al. 1991). Maturity stage can also affect the vitamin C content. Marı´n et al. (2009) found that green peppers grown under low irrigation frequency had similar content of vitamin C to red fruits and only the highly irrigated green fruits showed lower content as compared to red ones. Water consumption increases with increasing solar radiation, temperature and wind speed and decreases with increasing relative humidity. In other words, water consumption is a combined result of weather conditions for a given plant (Ünlükara et al. 2015).

4.4.4. Changes in tocopherols

The on-line operation of a fluorescence detector in addition to DAD in the HPLC technique allowed for the separation and determination of tocopherols in a single run (Figure 11). The tocopherol fraction was found to consist of α-tocopherol, the only biologically active form of vitamin E, and its esterified and oxidized derivatives, with modest amounts of ϒ- and β- tocopherols (Appendix 8). The fluorescently active α-tocopherol hydroquinone was found as the dominant oxidized derivative of α-tocopherol, which is generated by the reduction of quinones by active hydrogen donors such as vitamin C and phenols. The ratio of α-tocopherol/α-tocopherol hydroquinone (α-Toc/α-TocHQ) is of great importance from plant physiology point of view since it shows the real state of redox potential in pepper fruits and product. The ratio higher than 1 means low oxidation stress, and the ratio lower than 1 indicates the high oxidation stress in the plant products.

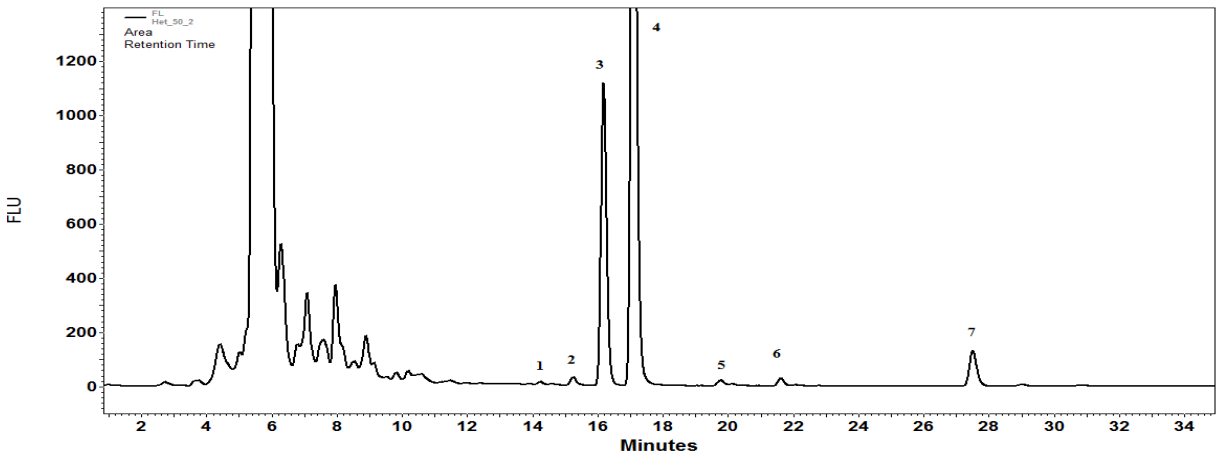

Figure 11: HPLC profile of chili pepper tocopherol separated simultaneously with carotenoids on C18, 3u, 240 x 0.46 mm column with gradient elution of (B) Acetonitrile-isopropanol-methanol in (A) methanol-water and FL detection at ex: 290 and Em:325nm. Peak identified as 1: γ-tocopherol, 2: β-tocopherol, 3: α-tocopherol quinone, 4: α-tocopherol, 5: γ-tocopherol ester, 6: β-tocopherol ester, 7: α-tocopherol ester. For more details, see text.

Among different analogs, only α-T has vitamin E activity (Azzi, 2019). As concerns the α-TQH_2, it has been shown to be the most efficient non-vitamin E prenyllipid antioxidant quenching and scavenging the singlet oxygen radicals generated in liposomes (Kruk, et al., 2016). Some studies have been conducted to study the biological role of α-T esters and their contribution to the bio accessibility of vitamin E. Most of these studies focused on the possibility of adsorption and hydrolysis by lipolytic enzymes of such esters on digestion in the intestinal Caco-2 cells (Brisson, et al. 2008; Cuerq, et al. 2021). It has been found that the types of carrier oil, ester moiety and presence of some poly saccharides affect the extent of adsorption, and hydrolysis of α-T esters and release of α-T in the cells (Lauridsen, et al. 2001; Yang and McClements, 2013; Cuerq, et al., 2021). Therefore, the focus was only on the major tocopherol analogs like α-Toc, α-Toc HQ, and α-Toc-Es.

The change in the concentration of tocopherols was found to be dependent on the variety and climate variables. During the 2019 cultivation season (Table 9), there was a great change in the concentration of the major tocopherols as a function of harvest time. The content of α-Toc tended to increase from 1st to 4th harvest with all WSTs in HET and UNIK. What altered from the tendency was that in HET at the 4th harvest a marked decrease happened with 0% WS and a negative effect of 100% WS was evident at the 1st harvest of UNIK.

Table 9: Effect of harvesting time and WS treatments on tocopherol compounds during 2019. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Tocopherol	WST/ Cultivar	1st harvest	2nd harvest	3rd harvest	4th harvest
α-Toc HQ					
	HET				
	0%	11.6±2.85Aa	36.2±6.32Bb	25.2±5.13Ba	28.2±7.95Ba
	50%	7.8±1.35Aa	23.0±3.25Ba	28.4±1.64BCa	34.5±7.20Ca
	100%	14.6±9.75Aa	29.0±2.08Bab	32.6±4.42Ba	35.0±2.82Ba
	UNIK				
	0%	12.4±2.21Aa	30.2±17.00ABa	27.0±16.00ABa	41.0±4.00Ba
	50%	12.3±2.33Aa	29.0±6.36Ba	42.0±6.21Ca	49.1±.27Ca
	100%	11.8±2.04Aa	39.3±6.05Ba	47.4±6.90Ba	34.4±21.82ABa
	UNIJ				
	0%	1.8±1.26Aa	8.2±1.04Ba	10.3±4.31Ba	5.7±2.00ABab
	50%	2.5±0.64Aa	7.0±1.67Ba	12.4±3.00Ca	7.20±0.93Bb
	100%	2.3±1.12Aa	8.8±1.87Ba	7.8±1.80Ba	3.5±2.10Aa
	HAB				
	0%	1.5±1.46ABa	2.6±1.43Ba	1.4±1.31ABa	
	50%	0.8±0.54ABa	1.9±1.32Ba	2.0±0.83Ba	
	100%	0.8±0.17Aa	2.3±1.00Aa	4.0±4.73Aa	
α-Toc					
	HET				
	0%	26.5±4.48ABa	58.1±7.16Ca	46.1±13.1BCa	13.3±1.27Aa
	50%	26.2±3.52Aa	44.1±7.15Ba	51.0±11.30Ba	69.0±1.71Cb
	100%	24.9±11.1Aa	48.7±11.53Ba	48.3±8.00Ba	64.5±3.31Bb
	UNIK				
	0%	33.6±7.54Ab	40.0±14.48Aa	47.3±12.48Aa	58.0±15.42Aa
	50%	22.9±5.43Aab	30.6±6.91Aa	45.3±9.62Ba	60.0±3.39Ca
	100%	15.4±4.00Aa	43.1±14.00Ba	38.2±14.05Ba	58.8±2.68Ba
	UNIJ				
	0%	1.4±2.01Aa	50.4±5.06Ba	50.0±19.00Ba	19.4±23.01ABa
	50%	5.1±3.05Aa	44.7±3.98Ba	50.0±7.18Ba	36.5±22.64Ba
	100%	6.0±5.61Aa	38.7±9.64Ba	29.0±6.28Ba	6.6±11.84Aa
	HAB				
	0%	0.1±0.03Aa	1.2±0.75Ba	0.2±0.13Aa	
	50%	0.1±0.05Aa	0.7±1.10ABa	1.7±0.78Ba	
	100%	0.2±0.04Aa	0.2±0.04Aa	1.5±1.51Aa	
αToc ester					
	HET				
	0%	1.3±0.33Aa	6.0±1.43Ba	6.1±1.28Ba	7.4±2.04Ba
	50%	1.6±0.37Aa	3.6±0.65Ba	6.3±0.95Ca	14.4±1.41Db
	100%	1.6±5.08Aa	4.0±1.65Ba	5.3±0.37Ba	10.3±1.20Ca
	UNIK				
	0%	2.0±0.75Aa	3.9±1.35ABa	6.5±0.58Ba	12.5±1.90Ca
	50%	1.8±0.62Aa	3.8±1.2ABa	6.0±1.14Ba	10.6±3.06Ca
	100%	1.1±0.20Aa	3.9±1.55Ba	5.5±0.78Ba	10.1±0.52Ca
	UNIJ				
	0%	0.2±0.10Aa	2.5±0.20ABb	5.2±1.44Cb	4.3±1.80BCab
	50%	0.4±0.10Aa	2.3±0.10Bb	4.2±0.66Cab	5.7±0.67Db
	100%	0.3±0.17Aa	1.7±0.34ABa	3.0±0.50Ba	2.8±1.34Ba
	HAB				
	0%	ND	1.4±0.79Ba	0.4±0.20Aa	
	50%	ND	0.7±0.80ABa	1.5±0.15Ba	
	100%	ND	1.0±0.62ABa	1.5±1.00Ca	

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test.

In the highly pungent UNIJ, a different response was noticed for vitamin E towards harvest and WSTs. The maximum level was found at the 2nd and 3rd harvest. The water stress with50% and 100% WS caused a significant increase in the vitamin content at the 1st harvest, while at the other harvest an inverse response was observed.

In the case of HAB, the peppers contained the lowest level of α-Toc at all harvests and there was no clear tendency of changes as a function of WSTs. The esterified α-Toc tended to respond to harvest and WSTs by similar way to that exhibited by the free form of vitamin E.

The most interesting response to harvest and WSTs was found for α-TocHQ, which showed a many-fold increase in its amounts at the 2nd, 3rd and 4th harvests. When related the content at these harvests to that determined at the 1st one an increase of 2.4-4.4 folds for HET, 2.4-4.0 folds for UNIK, 1.5-3.2 for UNIJ and 1.7-5.0 for HAB was found with slight positive or negative effect of the WSTs, mainly with 50%. These results confirmed the great influence of the climate variables on the oxidation stress that causes the oxidative degradation of both free and esterified form of vitamin E.

The results on tocopherols in pepper fruits harvested in 2020 are shown in Table 10. and detailed in Appendix 8. For α-Toc-HQ in HET, the harvest period had no effect with the 0% WST. With 50% treatment the significantly ($p<0.05$) higher level for the same compounds was determined at the 2nd harvest. The lowest value for this compound (18.80±0.99 μg /g fwb) was determined in HET at the 2nd harvest with 100% WST.

In UNIK, different response to harvest and WST was shown. The late harvest caused the content of α-Toc-HQ to significantly increase ($p<0.05$) as compared to that determined at the 1st and 2nd harvests regardless of the level of WS in contrast to HET, where the water stress (100%) resulted in a marked decrease in the concentration of such derivative.

In case of the highly pungent cultivars, the late harvests either dramatically decreased as took place in UNIJ or significantly increased ($P<0.01$) the content of such compound. These results affirmed that the climate conditions at the late harvest can affect by different ways the biochemical redox potential depending on the genetic factors and to some extent on the water supplies. The increased level of α-Toc-HQ in HAB is evidence on the activation of vitamin E oxidation at the cool weather of the late harvests via activation of the relevant enzymatic systems.

Table 10: Effect of harvest time and WS treatments on tocopherol compounds during 2020. The means are expressed in µg/g fresh weight base ± S.D (n = 4).

Tocopherol group	WST/ Cultivar	1st harvest	2nd harvest	3rd harvest
α-Toc-HQ				
	HET			
	0%	38.01 ± 3.43 Ab	38.04 ± 2.93 Ab	44.87 ±4.50 Ab
	50%	21.60 ± 5.64 Aa	35.58 ± 4.52 Bb	29.37 ±2.81 ABa
	100%	34.98 ± 3.17 Bb	18.80 ± 0.99 Aa	34.83 ±3.34 Ba
	UNIK			
	0%	29.57 ± 2.58 Aa	48.14 ±7.13 Ba	49.22 ±2.26 Ba
	50%	40.98 ± 4.89 Ab	41.95 ±5.30 ABa	54.65 ±6.03 Ba
	100%	38.01 ± 3.43 Aab	38.81 ±3.47 Aa	49.79 ±1.61 Ba
	UNIJ			
	0%	40.66 ± 1.21 Ba	9.02 ±0.89 Ab	9.42 ±0.47 Ab
	50%	32.18 ± 8.41 Ba	9.97 ±0.82 Ab	8.73 ±1.72 Aab
	100%	32.70 ± 0.80 Ba	6.01 ±0.74 Aa	6.03 ±1.15 Aa
	HAB			
	0%	1.39 ± 0.16 Aa	11.08 ±1.57 Ba	13.07 ±1.61 Ba
	50%	1.85 ± 0.58 Aab	11.08 ±1.79 Ba	13.43 ±2.01 Ba
	100%	3.53 ± 0.42 Ab	12.64 ±2.80 Ba	14.51 ±0.29 Ba
α-Toc				
	HET			
	0%	78.02 ± 3.55 Bb	75.54 ±1.53 ABb	66.74 ±4.99 Aa
	50%	67.82 ± 2.69 Aa	67.24 ±3.53 Aa	70.95 ±4.46 Aa
	100%	73.83 ± 2.67 Aab	67.82 ±2.69 Aab	73.83 ±2.67 Aa
	UNIK			
	0%	52.26 ± 4.50 Aa	78.10 ±2.99 Bb	69.50 ±8.59 Ba
	50%	81.39 ± 6.40 Bb	68.66 ±1.47 Aa	63.25 ±3.07 Aa
	100%	78.02 ± 3.55 Bb	74.95 ±4.39 Bab	57.92 ±3.04 Aa
	UNIJ			
	0%	77.72 ± 2.74 Bb	60.86 ±0.94 Aa	72.52 ±2.17 Bb
	50%	64.99 ± 6.71 Aa	66.35 ±1.03 Ab	63.24 ±4.84 Aa
	100%	64.65 ± 0.69 Aa	69.45 ±2.17 Bb	64.07 ±2.25 Aa
	HAB			
	0%	0.52 ± 0.03 Ab	36.38 ±6.90 Ba	34.70 ±2.74 Bab
	50%	0.16 ± 0.02 Aa	31.43 ±7.52 Ba	33.14 ±1.22 Ba
	100%	0.56 ± 0.24 Ab	32.97 ±2.60 Ba	39.97 ±3.50 Cb
α-Toc-Es				
	HET			
	0%	5.77 ± 1.87 Aa	5.47 ±0.90 Aa	11.58 ±1.16 Bb
	50%	5.71 ± 0.28 Aa	5.17 ±0.66 Aa	8.89 ±0.77 Ba
	100%	5.31 ± 0.59 Aa	6.08 ±0.39 Aa	12.31 ±0.59 Bb
	UNIK			
	0%	3.27 ± 0.07 Aa	6.40 ±0.29 Bb	11.48 ±0.93 Cb
	50%	5.57 ± 0.94 Aa	5.43 ±0.44 Aa	9.61 ±0.32 Ba
	100%	5.77 ± 1.87 Aa	6.66 ±0.20 Ab	8.20 ±0.48 Aa
	UNIJ			
	0%	5.80 ± 0.30 Bb	3.07 ±0.24 Aa	9.22 ±0.60 Cb
	50%	3.90 ± 0.71 Aa	3.57 ±0.13 Aa	7.62 ±0.61 Ba
	100%	4.63 ± 0.31 Bab	3.74 ±0.38 Aa	7.74 ±0.30 Ca
	HAB			
	0%	0.00 ± 0.00 Aa	1.35 ±0.28 Ba	1.76 ±0.21 Bab
	50%	0.00 ± 0.00 Aa	1.20 ±0.12 Ba	1.93 ±0.12 Cb
	100%	0.41 ± 0.07 Ab	1.18 ±0.22 Ba	1.49 ±0.16 Ca

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test

As also shown in Table10. that the biologically active form of vitamin E (α-Toc) slightly responded to the WS and harvesting time in all examined cultivars except HAB, in which the abiotic factors of the late harvests significantly increased the concentration of the vitamin ($P<0.001$). As concerns the effect of WST, there was a slight but significant decrease in the content because of the increased WS.

The content of the esterified form vitamin E, it is repeatedly evident that the climate of the last harvest is favorable for its biosynthesis since there was a significant increase in all cultivars studied ($P<0.01$). What unexpected was that the level in UNIJ at the 2nd harvest was the lowest. Further investigation is needed to know the real reason for such change. As regards the response of vitamin E ester to the WST, it can be said that it was slight and cultivar dependent.

The total amount of vitamin E in plants may vary by variety Krauß et al. (2020), studied a tocopherol in different varieties of chili pepper and found that habanero (*C. annuum*) has less tocopherol groups than the others pepper varieties that the authors studies. A similar result was observed in our research with habanero, in both years of research the habanero has the lower concentration of tocopherols for all harvest periods. Matsufuji, et al. (2007) research the content of α-tocopherol in sweet peppers in different color and found that as the genotype and the mature of the pepper matures, have higher level of α-tocopherol. The literature has lack of research about the WS, and the vitamin E compounds in chili peppers, most of the research with chili and fruits and vegetables in general focus more on the general content of α-tocopherol. Studies with cherry tomatoes showed that the irrigation system decreased α-tocopherol and increased γ-tocopherol and the content of total tocopherols is higher in non-irrigation system according to Pék et al. (2014).

4.5. Impact of drying methods on the phytonutrient content

4.5.1. Impact on Capsaicinoids

Table 11 shows the variations in the total capsaicinoids content and loss in various hybrids as a function of drying treatments. The capsaicinoid concentration of the examined hybrids' raw materials varied greatly, ranging from the lowest (3.540.28 71mg.g^{-1}dwt) in UNIK to the highest (31.472.71 mg. g^{1}dwt) in Unijol.

Table 11: Effect of drying method and conditions on the concentration and retention of total capsaicinoid in the dried peppers of the new hybrids.

	Drying treatments			
Cultivars	Before drying	Natural	Thermal 60°C	Thermal 90-25°C
		Concentration mg. g-1		
Hetényi	4.56±0.25a	3.96±0.44ab	2.68±0.37c	3.58±0.13b
Unikal	3.54±0.28a	1.61±0.09b	1.41±0.10b	1,25±0.09b
Unjol	31.47±2.71a	29.14±2.41a	21.11±1.18b	22.44±2.27b
		Retention %		
Hetényi	100	86	58	78
Unikal	100	46	40	35
Unijol	100	93	67	71

The same letter shows no significant difference between drying methods in the content of the total capsaicinoids according to Tukey's HSD post hoc test (at $P<0.05$*).*

UNIJ, which had the highest amount of capsaicinods, had the best stability, with retention ranging from 67 to 93 percent, while UNIK, which had the lowest level of capsaicinods, 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.

In the instance of HET, drying at 60°C for 30 hours yielded the least quantity of capsaicinoids preserved. Because the raw materials were not pretreated to inactivate the enzyme peroxidase, the high stability of capsaicinoids in naturally dried HET and UNIJ, could be attributed to the fact that they were whole pods without shredding or mincing, making it less likely for the enzyme to meet its substrates. Autoxidation is most likely to blame for the modest deterioration of capsaicinoids in naturally dried HET and UNIJ. The low stability of capsaicinoids during drying of the UNIK cultivar requires more investigation.

The principal capsaicinoids responded similarly to the total (Table 12), with the highest content in the raw materials' dry matter and the lowest in pods dried at 90-25°C. The concentration of NNDC and NCAP as intermediary metabolites of capsaicinoid production was unclear (Appendix 9). NCAPS was relatively stable in all hybrids when dried, and the highest amounts of NNDC were

found in naturally dried and thermally dried at 60°C products. The level of iso- and homo-structured derivatives was altered by drying conditions, with iDC being the most vulnerable. In the different cultivars, the proportions of the main capsaicin, dihydrocapsaicin, and nor-dihydrocapsaicin were 53-63 percent, 32-36 percent, and 2-7 percent of the total capsaicinoids, respectively. These ratios are like those discovered by Bianch and Scalzo (2018), but they differ significantly from those discovered by Topuz et al. in the chili pepper (2011). The ratio of CAP to DC, which ranged between 1.21 and 1.48 in UNIK, 1.60 and 1.68 in HET, and 1.82 and 2.10 in UNIJ, differed from the results of the following study. Topuz et al. (2011) measured 0.91.0.92 for Turkish chili pepper and these ranges are significantly higher. The highest levels of DC homo derivatives were found in the raw materials, then declined little or remained unchanged in the dried products. Unfortunately, most of the research concentrated on mainly CAP and DC, with little attention paid to the lesser compounds.

Table 12: Effect of drying methods and conditions on the concentration (µg.g^{-1}dwb) of the individual capsaicinoids of peppers from different hybrids.

	Drying treatments			
Capsaicinoids	**Natural**	**Batch 60°C**	**Gradual 90-25°C**	**Before drying**
	HET			
NDC	165.00±6.04c	224.66±12.52b	198.13±36.93b	250.44±42.29a
CAPS	1929.78±192.27b	2223.26±201.57b	1870.17±245.86b	2689.65±312.67a
DC	1173.59±99.04b	1330.68±127.57b	1162.71±185.50b	1771.91±378.34a
	UNIK			
NDC	122.39±17.63b	128.02±9.83b	114.45±5.97b	234.70±2.77a
CAPS	1342.58±115.57c	928.57±98.10b	857.60±121.95b	1858.82±162.75a
DC	902.74±71.20b	655.89±68.86b	707.21±41.01b	1283.25±108.42a
	UNIK			
NDC	706.32±100.37b	698.18±31.82b	653.07±87.07b	968.85±58.89a
CAPS	14948.28±1569.49c	12361.61±726.37b	13158.03±219.38bc	19866.64±373.04a
DC	8005.78±1187.21b	6823.49±270.53b	6286.62±526.17b	10037.05±406.62a

The same letter shows no significant difference between drying methods in the content of the individual capsaicinoids according to Tukey's HSD post hoc test (at $P<0.05$).

4.5.2. Impact on Carotenoids

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.

Table 13 demonstrates how the total carotenoid content changes as a function of drying time under various settings. It was clear that the stability of the major phytochemicals in the different cultivars differed during the drying process. Although HET had the highest carotenoid content, UNIK had the highest color loss during both natural and thermal drying. In comparison to the content in the raw material before drying, pigment retention was 43-53% for HET cultivars and 52-77% for UNIK cultivar. The greatest loss was seen in both cultivars when the drying temperature was gradually reduced from 90°C to 25°C in 10 hours. The negative impact of hot air drying on carotenoid stability is obvious in the present study and are consistent with that of Vega-Galvez et al. (2009), who found that drying at high temperatures between 80 and 90°C resulted in the greatest loss of ASTA values.

Table 13: Change in content of total carotenoids from new chili cultivars as a function of different drying treatments.

	Drying treatments			
Cultivars	Raw before drying	Natural	Thermal 60°C	Thermal 90-25°C
		Concentration mg. g^{-1}		
Hetényi	6.05±0.27a	3.23±0.07b	2.80±0.19c	2.60±0.34c
Unikal	4.46±0.34a	3.46±0.28b	2.64±0.440c	2.31±0.27c
Unjol	4.10±0.25a	5.65±0.19b	3.38±0.05c	3.13±0.16c
		Retention %		
Hetényi	100	53	46	43
Unikal	100	77	59	52
Unijol	100	138	82	76

The same letter shows no significant difference between drying methods in the content of the total carotenoids according to Tukey's HSD post hoc test (at $P<0.05$).

Natural drying increased the total carotenoid concentration in the highly pungent UNIJ hybrid by 38 percent as compared to the original concentration in peppers before drying. This rise is most likely owing to the continuing of carotenogenic pathways catalyzed by the relevant enzymes, which remain active until water activity decreases to very low levels. Some sweet spice red peppers have shown a similar rise when dried naturally or thermally at low temperatures (Minguez-Mosquera; and Hornero-Mendez, 1994; Ergunes and Tarhan, 2006). Thermal drying resulted in a loss of 18% and 24% for UNIJ dried at 60°C and 90-25°C temperatures, respectively. These findings suggest that the thermal drying used in this investigation may have inactivated carotenogenic processes and had a little detrimental impact on carotenoid stability in such a hybrid.

The carotenoid concentration of UNIJ samples dried by the different methods was substantially higher ($P<0.01$) than the carotenoid content of dried products from HET and UNIK samples, which did not differ significantly from each other in their carotenoid content. The high stability of carotenoids in the UNIJ cultivar may be linked to the high amount of capsaicinoids, which may protect carotenoids from thermal and oxidative degradation during the drying and post-drying processes (Daood et al. 2006; Schweiggert, Kurz, Schieber, 2007). According to Topuz et al. (2011), Adamu, Ariahu, Igbabul, (2021), and Villa-Rivera and Ochoa-Alejo (2020) naturally dried peppers from all the cultivars studied retained.

Table 14 shows the effects of various drying procedures on the on the most important groups, while Appendix 10 shows the effect on all carotenoid groups.

The concentration of free red xanthophylls dropped dramatically because of drying in the HET cultivar, with the lowest retention seen in naturally dried pods. Because it behaved inversely to mono- and di-esters, it's thought that the over-ripeness process in this cultivar continued to some extent during natural drying. Márkus et al. 1999; Gnayfeed et al. 2001; Bonaccorsi et al. 2016; Mercadante et al. 2017) have found that the over-ripeness of red-colored spice peppers is characterized by ongoing carotenoids synthesis and an increase in the rate of esterification of xanthophylls with fatty acids at the expense of the unesterified ones (Márkus et al. 1999; Gnayfeed et al. 2001; Bonaccorsi et al. 2016; Mercadante et al. 2017). In naturally dried, dried at 60°C, dried at 90-25°C, and raw materials, the ratios of monoesters/unesterified and diesters/unesterified were 4.71, 1.05,1.45, 2.31, and 32.10, 4,45, 4.13,6.9, respectively. The highest ratio of esterified/unesterified red xanthophylls assessed in naturally dried HET pepper pods is a strong indication of over-ripeness during natural drying.

Table 14: Change in the carotenoid groups content (µg.g^{-1} dwb) as a function of natural and thermal drying of the new hybrids at different conditions.

	Drying treatments			
Carotenoid groups	**Before drying**	**Natural**	**Thermal 60°C**	**Thermal 90-25°C**
		HET		
Total Red	4026.39±163.38a	2291.29±61.13b	1999.56±110.41b	1868.76±213.76b
Total Yellow	1600.85±109.41a	1047.37±54.86b	943.14±22.07b	722.11±83.19b
Red/Yellow	2.52±0.07a	2.19±0.16b	2.12±0.14b	2.56±0.15a
β-carotene	467.02±29.46a	275.96±25.80b	239.58±10.11c	201.15±28.04c
Total pro Vitamin A	604.46±41.60a	328.35±30.99b	313.41±23.40b	296.29±36.13b
		UNIK		
Total Red	2514.47±205.22a	2466.09±180.19a	2137.33±227.75a	1574.92±192.10b
Total Yellow	1492.12±132.35a	1401.44±128.94a	958-68±78.70b	876.82±95.51b
Red/Yellow	1.68±0.02a	2.26±0.08b	2.23±0.09b	1.78±0.08a
β-carotene	452.16±26.34a	468.29±64.29a	239.58±10.11b	210.15±12.81b
Total pro Vitamin A	606.06±36.17a	580.99±79.95a	307.21±20.14b	265.17±18.16b
		UNIJ		
Total Red	2039.32±209.55a	3726.20±141.17b	1959.58±83.70a	1948.27±117.96a
Total Yellow	1070.50±41.30a	2215.79±127.27b	1024.97±71.11a	936.68±55.98c
Red/Yellow	1.90±0.12a	1.68±0.08b	1.91±0.05a	2.08±0.06ac
β-carotene	541.52±51.82a	765.29±30.24b	397.33±12.85c	348.68±16.01c
Total pro Vitamin A	579.04±49.59a	934.79±40.34b	531.64±25.65a	437.02±24.22c

MEs= monoesters, DEs = di-esters.
The same letter shows no significant difference between drying methods in the content of the carotenoid groups according to Tukey's HSD post hoc test ($P<0.05$).

Different kinds of red xanthophylls responded differently to thermal drying conditions. Unesterified, monoesters, and di-esters suffered the greatest losses of 33%, 68%, and 61 percent, respectively. These findings contradict the fact that xanthophyll esterification improves their resistance to heat and oxidative degradation (Pérez-Gálvez and Minguez-Mosquera, 2005; Daood et al. 2006). The presence of unsaturated fatty acids in the lipid moiety of some yellow- and red-colored esters from chili peppers (Dauh et al. 2021) may explain why esterified xanthophylls are less stable after heat drying than unesterified ones. According to Pérez-Gálvez and Minguez-Mosquera, (2005), when unsaturated fatty acids esterify to xanthophylls, their antioxidant activity is reduced due to the propagation of the radical chain. Nonetheless, Kim, Park, and Hwang (2004) reported that, depending on the drying technique and storage temperature, free capsanthin has the same or higher stability as mono or di-esters, with monoesters being the least stable.

Yellow-colored xanthophylls responded to natural drying in a similar fashion to red pigments, especially in terms of free, mono-, and diester levels. The lowest content of free yellow xanthophylls

and the highest content of total yellow pigments in naturally dried peppers corresponded to the statement that biosynthesis of yellow and then red xanthophylls occurs during natural drying of many sweet and pungent pepper species, producing more carotenoids that esterify with fatty acids (Gnayfed et al. 2001; Tupoz et al. 2011). The lowest content of mono- and di-esters was obtained using thermal drying methods, with a significant difference between them ($p=0.01$) in only the concentration of monoesters of yellow xanthophylls. The maximum degradation (66-71 percent) was obtained for both mono- and di-esters of yellow xanthophylls under high and variable temperature drying (90-25°C), showing that their stability to high-temperature drying is worse than that of red xanthophylls. Chili peppers dried at 90-25°C had the highest ratio of red/yellow carotenoids (2.560.15), which corroborated the previous findings. Except for β-cryptoxanthin, the main groupings of yellow pigments responded to heat and natural drying in the same way that total yellow and red/yellow ratio did. The retention of β-cryptoxanthin in peppers dried at 60°C and naturally was 65.6 percent in the 90-25°C treatment, compared to 50.6 percent and 37.8 percent in peppers dried at 60°C and naturally, respectively. The significant stability of β-cryptoxanthin may be owing to high temperature treatment inhibiting the enzymes responsible for its destruction and/or physical protection offered by the primary ingredients against thermal degradation. In terms of the influence of drying on provitamin A carotenoids, it was clear that their high retention necessitated the use of low temperature drying, regardless of the drying time. Topuz et al. (2011) found that the level of yellow pigments in the naturally dried product is higher even than that in the raw material, which is compatible with the reaction of yellow-colored groups in HET cultivar (puree). The level of provitamin A molecules in naturally dried and raw materials did not differ significantly in this study. Differences in genotypes and drying conditions, particularly the physical state of the raw material used, can explain this discrepancy.

With high temperature-short time drying, the reaction of unesterified and esterified carotenoids in UNIK cultivar showed similar tendencies to those seen in HET, with an unusual change in red xanthophyll stability, which was substantially lower ($p<0.05$) than that of yellow ones. As a function of drying temperature, the amount of lost red and yellow pigments was 940 and 616 g from 1 gram of dry matter, respectively. Treatments with low temperature-long time resulted in a high level of red carotenoids, causing the ratio of red to yellow to increase from 1.68 to 2.26. The total carotenoids and specific groups in UNIK were measured in naturally dried chili peppers, confirming Topuz et al. (2011)'s claim that carotenoid production continues during natural drying. However, the concentrations of both lutein and zeaxanthin were larger with the high temperature drying treatment than with the other treatments, and they reacted inversely to other yellow-colored compounds

including β-cryptoxanthin and β-carotene. These findings are particularly interesting from a biological and human nutrition standpoint, as lutein and zeaxanthin play a significant role in macular membrane visual activity (Widomska and Subczynski, 2014).

The naturally dried product of the exceedingly pungent hybrid UNIJ, which was generated by cross breeding red habanero and Hungarian traditional pungent spice red pepper, shows a significant rise in the content of total and individual groups. This significant rise suggests that it takes longer for a cultivar to reach technical ripeness, when carotenoid synthesis is complete, than it does for other cultivars. The concentration of total red and total yellow, which exhibited marked stability during drying of such hybrids, did not change significantly across different thermal drying procedures. The lowest quantities of physiologically active and provitamin A carotenoids were detected in pods dried at 90-25°C.

The red-to-yellow ratio determined for the dried products from the three cultivars studied ranged from 1.68 to 2.56, which is somewhat higher than the values published by Topuz et al. (2011) for jalapeno chili and within the range reported by Minguez-Mosquera, Pérez-Galvez and Garrido-Fernandez (2000). Because red xanthophyll has been shown to be more stable than yellow xanthophyll, the dried products of the new hybrids exhibit an amazing red hue and may have a high storage stability (Minguez-Mosquera, Pérez-Galvez and Garrido-Fernandez 2000; Daood et al. 2006).

It is well known that the content of cis isomers of carotenoids increases as a result of heat treatments (in thermal drying) and chemical variables such light, oxygen, and enzymes (in natural drying) (Namitha and Negi, 2010). The production and stability of the cis isomers varies significantly between the hybrids. thermally dried samples of UNIK, for example, had the highest level of cis-β-carotene, but HET and UNIJ's thermally dried samples had significantly lower quantities ($P<0.01$). The highest concentration of cis-β-carotene was found in naturally dried UNIJ product, which contained around 6.3 times more than the products from the other cultivars. The genotype-dependence of enzyme-catalyzed all trans to cis isomerization of carotenoids is obvious from these findings.

4.5.3. Impact on Tocopherols

The presence of α-TocQH2 and the absence of α-TocQ indicated the presence of extremely active reducing agents in the various products of spice chili peppers that convert quinone to hydroquinone. Although the vitamin E activity of such derivatives has yet to be shown, certain research has highlighted the biological significance of TocQH2 as a bio-antioxidant (Kruk, et al. 2016). The

biological significance of esterified vitamin E is based on its breakdown by lipolytic enzyme and adsorption in intestinal Caco-2 cells, according to certain studies (Yang and McClements, 2013; Cuerq, et al. 2021).

Table 15 shows the response of total tocopherols in the three chili peppers to drying conditions, as well as the percentage of tocopherols retained in the dry products. 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.

Table 15 Change in content (µg. g^{-1} dwt) and retention (%) of total tocopherol as a function of drying treatments of different chili cultivars.

	Drying treatments			
Cultivars	Before drying	Natural	Thermal 60°C	Thermal 90-25°C
	Concentration µg. g^{-1} dwt			
Hetényi	878.38±52.95a	630.27±28.00b	696.74±20.81b	634.77±56.25b
Unikal	1032.68±108.20a	759.16±58.48c	655.22±40.10b	662.07±19.40b
Unjol	613.07±17.69a	508.73±26.26b	569.22±33.80b	550.26±22.12b
	Retention %			
Hetényi	100	72	79	72
Unikal	100	73	63	64
Unijol	100	83	92	89

The same letter shows no significant difference between drying methods in the content of the total tocopherol according to Tukey's HSD post hoc test (at $P<0.05$).

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.

Despite a 22-35 percent loss in vitamin E after drying, the dried products of the hybrids studied still contain substantial levels of vitamin E. The amount of vitamin E in a gram serving of freshly produced powder ranged from 375 to 408 µg, accounting for 2.5-2.7% of the 15 mg recommended daily allowance (RDA) for the vitamin (Rizvi et al. 2014).

Table 16: Change in the content (µg.g-1dwt) of tocopherol analogs from different chili hybrids as a function of different drying treatments

	Drying Treatments			
Tocopherols	**Natural 25°C**	**Thermal 60°C**	**Thermal 90-50°C**	**Before drying**
	HET			
γ+β-Toc	3.42±0.23b	3.808±0.3b	3.55±0.55b	4.76±1.09a
α-Toc-HQ	128.14±6.10b	153.86±6.92c	126.69±8.32b	258.43±29.97a
α-Toc	377.27±18.71b	393.07±12.05b	375.92±23.44b	539.54±10.95a
α-Toc-HQ-Es	80.74±9.03b	109.43±7.92b	96.73±8.70b	5.601±4.88a
α-Toc-Es	40.68±4.20b	36.47±1.96b	31.86±6.29b	54.22±6.04a
	UNIK			
γ+β-Toc	5.45±0.84b	7.87±1.77c	5.81±0.63b	11.85±2.66a
α-Toc-HQ	214.99±31.00b	161.32±16.51b	189.21±8.15b	361.22±80.81a
α-Toc	408.60±24.58b	383.59±18.97b	364.69±13.02b	557.88±51.37a
α-Toc-HQ-Es	73.59±15.62b	67.33±12.14b	67.28±11.43b	5.37±1.27a
α-Toc-Es	56.50±6.29c	35.10±4.97b	35.07±3.50b	44.35±3.10a
	UNIJ			
γ+β-Toc	2.09±0.15a	2,12±0.33a	1.78±0.22a	1.77±0.14a
α-Toc-HQ	54.26±1.74b	66,47±7.68ab	57.28±4.16b	75.17±7.42a
α-Toc	403.01±12.08b	408.67±7.96b	396.23±8.12b	507.12±7.84a
α-Toc-HQ-Es	13.65±13.64c	61.89±14.29b	67.17±11.39b	3.39±0.32a
α-Toc-Es	35.70±1.00c	30.05±1.82b	27.78±1.45ab	25.60±1.89a

The same letter shows no significant difference between drying methods in the content of the individual tocopherol compounds according to Tukey's HSD post hoc test (at $P<0.05$).

5. CONCLUSION 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 climate-dependent 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.

- 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 high-temperature-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).

6. NEW SCIENTIFIC RESULTS

1) 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 post-harvest 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 R^2=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 β-carotene.
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.

7. SUMMARY

Introduction

An increasing interest is being given to chili peppers all over the world and especially in Hungary. In addition to the economic importance of chili peppers, they receive special interest in the fields of biology and human nutrition due to the presence of important phytochemicals highlighted in this study in capsaicinoids, carotenoids, vitamin C and tocopherol (vitamin E), which when ingested in our diet, bring benefits by having actions in the body as antioxidants, anti-inflammatory, anti-tumorigenic, preventing cancer, central nervous system, neurodegenerative diseases. Due to the climatic factors and the stress that these pepper plants can experience in open-air plantings, the plant has a metabolic system to adapt to minimum or maximum stress conditions when these affect its state in a stress alert situation. In this study we focused on the production of bell pepper in open field subjected to environmental factors and under water stress system. In parallel with this study, it was also focused on how the drying temperature factor can affect and preserve the phytochemical compounds when peppers are subjected to drying.

Materials and Methods

The experiment was conducted for two consecutive years under open cultivation conditions and was investigated during the production period effect of physiological factors and phytochemical responses of chili pepper cultivars under three different WS treatments. In the experiment dry pepper was subjected to drying 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. The experimental design used in both study periods 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). The physiological factors measured were relative chlorophyll content (expressed as SPAD values), chlorophyll fluorescence (Fv/Fm), canopy temperature and soil moisture. WS treatment was 0% or control (considering natural precipitation), 50% deficit irrigation and 100% optimal drip WS. The two years of experiment there was a significant difference in 2019 the mean temperature was 25.8 °C and the rainfall was 132.6 mm on 2020 the mean temperature was 28.5 °C and the precipitation was 473.6 mm. According to the climatic conditions and state of the fruits, there were 4 harvest periods in 2019 and 3 harvest periods in 2020. Analyzes performed on high performance liquid chromatography (HPLC) equipment according to each protocol.

Results and Discussion

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 variety least adapted to the climatic conditions of the study was the HAB species, which responded poorly to the effect of water supplementation and to climatic conditions in general. The UNIJ and UNIK cultures showed similar behavior for some compounds during the study period. In general, the marketable yield in 2019 was higher production for HET 0% (24.67±3.34 t/ha) and lower production for HAB 50% (3.36±0.47 t/ha), while in 2020 the highest production was for UNIJ 100% (33.58±1.55 t/ha) and the lowest for HAB 0% (9.03±3.09 t/ha). Overall, UNIJ had the highest production and HAB the lowest output in 2020. Regarding secondary phytonutrient compounds in fresh pepper, there was a variation between the two years, being different. Due to high rainfall in 2020 the treatment WS 0%, 50% and 100% were not significantly affected 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. The major capsaicinoids concentration (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) in 2019 was highest in the UNIJ variety peaking at 2743.1±429 µg/g, 2920.7±567.1 µg/g AND 269.5±37 µg/g respectively for capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. In 2020 UNIJ also showed the highest concentrations for capsaicin 2875.77 ±411.00 µg/g, dihydrocapsaicin 1341.31±135.23 µg/g and nordihydrocapsaicin. 176.14±12.93 µg/g. The total carotenoid was higher in HET during all four harvests in 2019 and lower concentration in HAB. On 2020 when the rainfall precipitation was higher no, the biggest difference was presented in the amount of total carotenoid between the varieties during the harvest period. The vitamin C in 2019 had higher concentrations for all varieties of the fourth harvest, with the highest concentrations occurring for HET 0% (4397.5±76.9 µg/g) UNIK (4184.0±286.2 µg/g) UNIJ (3842.3±96.7 µg/g) and HAB (3223.7±118.6 µg/g). In 2020, between the harvest periods and between the types of treatment, there were no significant differences and the maximum and minimum vitamin C values occurred in UNIK 0% (4855.63 ± 365.56 µg/g) and UNIJ 100% (1369.88 ± 198.90 µg/g) g). Based on our findings, α-tocopherol in 2019 had a variation between the maximum and the minimum according to the type of variety and harvest time 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% 2nd harvest As for HAB there were the lowest values for a-tocopherol being the maximum and minimum 1.7±0.78 µg/g in 50% and 0.1±0.03 µg/g for all water treatments of the 1st harvest. In 2020, the concentration of α -Toc had higher values when compared to the values of 2019, with the

maximum and minimum being 81.39 ± 6.40 µg/g in UNIK 50% and minimum for HAB 50% 0.16 ± 0.02 µg/g). For dry samples, Unijol. UNIJ, which had the highest amount of capsaicinoids, had the best stability, with retention ranging from 67 to 92 percent, while UNIK, which had the lowest level of capsaicinoids, had the lowest stability, with retention ranging from 35 to 45 percent. UNIK saw the greatest color loss after both natural and thermal drying, despite HET having the highest carotenoid content. Pigment retention was 43-53% for HET cultivars and 52-77% for UNIK cultivars when compared to the content in the raw material before drying. In our research about drying methodology other compounds also were studied and have different results as Yellow-colored xanthophylls responded to natural drying similarly to red pigments, especially in terms of free, mono-, and diester levels in general we could conclude that for carotenoids the high retention necessitated the use of low-temperature drying, regardless of the drying time. Most of all tocopherols, the highest concentration of tocopherol compounds, was found in the raw materials before drying and was reduced by natural and heat drying. In our research, we could evaluate various tocopherol compounds for drying treatments. Despite a 22-35% loss in vitamin E after drying, the dried products of the hybrids studied still contain substantial levels of vitamin E.

8. REFERENCES

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9. APPENDICES

Appendix 1: Data used for the identification of carotenoid compounds extracted from red chili pepper and analyzed by LC-DAD-MS procedure as described.

Peak	Rt	carotenoid ID	Maximum absorption λ			$[M+H]^+$
1	9.8	Capsorubin	446	478	511	601.2
2	10.4	5.6-diepikarpoxanthin	419	443	471	605.2
3	10.8	Capsanthin epoxide		472		601.5
4	11.2	Violaxanthin	418	438	468	601.4
5	11.8	Capsanthin		472		585.2
6	12.3	Antheraxanthin	421	447	476	585.4
7	13.4	Lutein	423	444	472	568.2
8	13.7	Zeaxanthin	425	451	478	568.3
9	16.2	cis-Zeaxanthin	419	445	474	568.4
10	16.9	β-cryptocapsin		454	481	568.2
11	18.3	cis-Zeaxanthin-C14:0	423	447	474	778.4
12	18.7	β-cryptoxanthin	426	451	480	567.4
13	19.1	Capsanthin epoxide C14:0		471		811.3
14	19.5	Capsanthin C14:0		473		795.4
15	19.9	β-cryptocapsin-C14:0	425	451	478	777.8
16	20.2	cis-capsorubin-C14:0	357	468	509	811.2
17	20.6	Capsanthin ME C16:0		472		823.4
18	21.3	Antheraxanthin C12:0	425	446	475	749.4
19	22.5	cis-Cryptocapsin ME	354	448	476	749.4
20	22.8	Zeaxanthin C16:0	426	451	480	792.2
21	23.5	Antheraxanthin C16:0	424	446	475	809.3
22	24.3	β-cryptocapsin C16:0	454	482	492	805.3
23	25.1	cis-Zeaxanthin	424	446	476	934.4
24	25.9	β-carotene	427	451	480	537.4
25	27.4	Capsorubin C14:0. C14:0	456	483	511	1022.4
26	27.8	cis-Capsanthin C12:0. C14:0	358	468	498	977.2
27	28.7	cis-Capsorubin C14:0. C14:0	357	468	509	1022.4
28	29.2	Capsorubin C14:0. C16:0		478	511	1049.4
29	29.8	Capsanthin C12:0. C14:1		474		975.2
30	30.7	cis-Capsorubin C14:0. C16:0	356	468	508	1049.4
31	32.7	Capsanthin C12:0. C16:0		473		1005.2
32	33.2	cis-Capsanthin C14:0. C14:0	358	468	490	1005.3
33	34.7	Capsanthin C14:0. C16:0		472		1033.4
34	35.9	cis-Capsanthin C14:0. C16:1		472		1031.4
35	36.0	cis-Capsorubin C14:0. C16:0	357	468	509	1049.3
36	37.4	Capsanthin C16:0. C16:0		473		1061.4
37	38.2	Zeaxanthin C14:1. C16:0	426	452	480	1014.2
38	39.1	cis-Capsorubin C16:0. C16:0	357	468	509	1077.4
39	39.8	Capsanthin C16:1. C18:0		473		1089.2
40	41.2	Zeaxanthin C16:0. C16:0	426	452	480	1045.4
41	44.4	cis-Zeaxanthin C16:0. C16:0	418	445	474	1045.3

Appendix 2: The average yield of the chili peppers cultivated in 2019 (n = 4; mean ± SD) based on fresh weight (t/ha)

Cultivar	WST	1st harvest	2nd harvest	3rd harvest	4th harvest	Total yield (t/ha)
HET	0%	12.75±2.01Bb	1.11±0.11Aa	9.38±0.96Bb	1.43±0.25Aa	24.67±3.34b
	50%	11.18±1.03Cb	2.37±0.24Ba	2±0.01Ba	0.4±0.05Aa	15.96±1.33a
	100%	8.67±0.81Ca	2.17±0.26Ba	8.74±2.77Cb	0.44±0Aa	20.02±3.84b
UNIK	0%	4.49±0.66Ba	1.65±0.18Aa	3.54±0.77Bb	1.14±0.3Aa	10.83±1.91a
	50%	13.99±2.29Cc	1.96±0.32Ba	1.2±0.22Ba	0.26±0.03Aa	17.4±2.85b
	100%	8.36±1.00Cb	1.83±0.15Ba	2.64±0.23Bb	0.41±0.05Aa	13.24±1.44a
UNIJ	0%	0.79±0.32Aa	2.14±0.28Ba	0.65±0.13Aa	0.17±0.04Aa	3.76±0.77a
	50%	3.17±1.02Bb	8.72±1.64Cb	8.16±1.12Cc	0.4±0.11Aa	20.45±3.89c
	100%	1.56±0.61Aa	3.99±0.52Aa	2.88±0.49Ab	1.19±0.75Aa	9.62±2.37b
HAB	0%	2.03±0.23Bb	1.79±0.26Ba	0.6±0.11Aa	4.41±0.6a	
	50%	0.39±0.06Aa	2.72±0.38Ba	0.25±0.03Aa	3.36±0.47a	
	100%	1.9±0.05Ab	12.57±1.44Bb	0.58±0.07Aa	15.04±1.56b	

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents water supply according to Tukey's HSD post hoc test

Appendix 3: The average yield of the chili peppers cultivated in 2020 (n = 4; mean ± SD) based on fresh weight (t/ha)

Cultivar 2020	WST	1st harvest	2nd harvest	3rd harvest	Total yield (t/ha)
HET	0%	7.23 ± 0.89Cb	5.10±0.93Ba	3.18±0.70Aa	15.51±2.03a
	50%	5.21±1.08Ba	6.02±0.43Ba	3.95±0.39Aa	15.81±1.04a
	100%	6.35±1.28Bab	7.02±0.25Bb	5.24±0.63Ab	18.61±0.90b
UNIK	0%	5.70 ± 1.53Aa	6.77±1.63Aa	6.73±0.72Aa	19.20±0.61a
	50%	6.00 ± 0.43Aa	7.03±0.37Aa	7.52±0.33Aab	20.55±0.78ab
	100%	7.64±0.32ABb	6.60±0.47Aa	8.27±0.47Bb	22.51±0.84b
UNIJ	0%	4.52 ± 0.58Aa	8.21±0.41Ba	10.21±0.68Ca	22.94±2.89a
	50%	5.52±0.18Aa	9.42±0.44Ba	11.04±1.21Cab	25.98±2.84b
	100%	9.43±0.32Ab	11.81±1.05Bb	12.34±0.42Cb	33.58±1.55c
HAB	0%	0.43 ±0 .12Aa	6.44±1.18Ca	2.16±0.47Ba	9.03±3.09a
	50%	0.62 ± 0.16Ab	6.42±0.32Ca	2.04±0.28Ba	9.08±3.02a
	100%	0.54±0.03Aab	6.40±0.16Ca	3.05±0.23Ba	9.99±2.94a

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test

Appendix 4: Effect of WS on capsaicinoid concentration in the various pepper cultivars for the 2019 growing season. The means are expressed in µg/g fresh weight base ± S.D (n = 4).

Capsai-cinoids	WST/ cultivar	1st harvest	2nd harvest	3rd harvest	4th harvest
NDC					
	HET				
	0%	56.5±4.7Ab	42.9±10.5Ab	63.0±14.8ABb	86.6±15.9Bb
	50%	36.2±1.6ABa	27.1±6.4Aa	41.1±9.3Ba	26.9±4.18Aa
	100%	37.8±3.2Ba	20.3±4.2Aa	23.1±7.1Aa	47.2±8.41Ba
	UNIK				
	0%	46.2±7.0BCa	18.2±2.1Aa	19.7±5.2ABa	64.5±24.9Cb
	50%	32.5±8.3Ba	17.8±2.9Aa	16.1±7.4Aa	19.7±5.6ABa
	100%	42.5±7.7Ba	13.8±2.25Aa	15.7±9.6Aa	30.6±5.5Ba
	UNIJ				
	0%	210.0±40.9ABa	150.7±21.9b	228.2±17.5Bb	350.0±56.3Cb
	50%	269.5±37.4Ba	117.5±7.9Aa	137.5±42.9Aa	239.5±29.3Ba
	100%	241.5±46.6Ba	78.3±14.6Aa	98.0±18.3Aa	284.5±33.5Bab
	HAB				
	0%		108.5±19.6Ab	146.1±8.3Bb	187.2±15.8Cb
	50%		63.87±8.3Aa	67.3±10.4Aa	105.8±15.7Ba
	100%		42.87±3.3Aa	61.25±10.5Ba	105.0±0.0Ca
CAP					
	HET				
	0%	584.1±19.1Cb	375.7±109.78ABa	298.2±65.7Aa	509.6±100.3BCb
	50%	458.6±22.5Ba	300.6±53.8Aa	312.7±77.6Aa	296.3±70.7Aa
	100%	514.8±95.0Bab	238.7±27.7Aa	258.3±72.9Aa	522.0±78.2Bb
	UNIK				
	0%	236.4±42.4Cb	99.4±18.3ABa	68.6±21.4Aa	147.8±55.7Ba
	50%	145.6±28.5Aa	131.6±17.3Aa	104.3±57.5Aa	113.9±34.9Aa
	100%	187.4±38.8Bab	98.2±16.5Aa	91.3±22.5Aa	105.8±13.7Aa
	UNIJ				
	0%	1763.5±46.5Aa	1526.5±56.8Bb	1936.3±216.5Ba	2743.1±429.8Ca
	50%	1992.5±109.4Aa	1900.8.3±207.4Aa	2099.3±404.3Ba	2507.0±354.2Ca
	100%	1902.8±172.7Aa	1514.3±139.1Aa	2014.2±301.5Aa	2217.2±231.1Aa
	HAB				

	0%		2744.3±317.0 Bab	2549.7±181.0 ABa	2315.2±171.4Aab
	50%		2969.7±162.9 Ab	2495.5±218.4 Aa	2943.5±411.2Ab
	100%		2392.2±262.6 Aa	2381.7±153.0 Aa	2202.3±441.9Aa
DC					
	HET				
	0%	329.7±18.5ABb	236.2±68.5Ab	260.0±73.2A Ba	376.7±75.2Bb
	50%	230.1±10.3Aa	163.1±33.2Aa b	207.7±56.8A a	158.9±37.8Aa
	100%	260.0±38.5Ba	123.9±19.9Aa	142.8±47.0A a	280.1±41.5Bb
	UNIK				
	0%	169.9±36.9Ab	232.4±311.3A a	64.5±19.8Aa	164.3±63.5Aa
	50%	106.7±21.7Aa	80.1±9.3Aa	79.9±48.1Aa	89.9±28.8Aa
	100%	135.4±18.7Bab	58.6±8.9Aa	61.6±21.3Aa	88.0±9.4Aa
	UNIJ				
	0%	798.8±24.5Ba	1091.6±146.1 Ab	1418.3±126.6 Aa	2131.3±244.7Bb
	50%	1161.7±223.1Ca	1071.3±113.4 Aa	1313.3±422.6 Ba	1463.8±453.9Ba
	100%	1046.2±125.5Ca	821.3±160.7A a	928.3±104.0 Ba	1066.6±126.4Ba
	HAB				
	0%		1323.8±155.8 Bc	1344.8±56.2 Bb	1080.6±76.1Ab
	50%		1034.25±99.4 Ab	979.1±47.0A a	1036.8±117.3Aab
	100%		742.8±75.9Aa	886.37±74.1 Aa	858.3±111.6Aa
HCAP					
	HET				
	0%	2.9±1.3Aa	1.6±0.3Aa	5.5±0.9Bb	8.3±1.3Cb
	50%	3.1±0.4ABa	1.7±0.7Aa	4.8±1.2Bb	2.0±0.85Aa
	100%	4.0±0.7Ca	1.4±0.00ABa	0.7±0.0Aa	1.9±0.35Ba
	UNIK				
	0%	1.3±1.5Aa	0.3±0.6Aa	ND	ND
	50%	ND	1.0±0.5ABa	0.2±0.5ABa	1.1±0.8Ba
	100%	0.5±1.1Aa	1.1±0.4Aa	ND	0.4±0.7Aa
	UNIJ				
	0%	1.7±3.5Aa	ND	ND	ND
	50%	6.1±1.7Ba	ND	0.3±0.5Aa	ND
	100%	1.7±2.0Aa	ND	0.3±.0.5Aa	ND
	HAB				
	0%		8.7±8.3ABa	25.5±3.6Cb	14.0±0.0Bb
	50%		5.2±2.0Ba	2.8±0.5ABa	11.4±3.3Cab
	100%		7.0±0.0Ba	3.3±0.2Aa	7.8±1.7Ba

iDC					
	HET				
	0%	5.7±1.4Ab	7.2±1.0Ab	20.7±1.7Cc	15.8±3.7Bb
	50%	3.3±0.7Aa	4.0±1.0Aa	3.3±0.7Bb	3.4±1.2Aa
	100%	2.9±1.0Aa	2.4±0.4Aa	2.9±1.0Aa	2.9±0.3Aa
	UNIK				
	0%	6.2±4.0Aa	3.9±1.0Ab	4.7±0.3Ab	13.1±4.21Aa
	50%	7.1±1.4Ba	2.6±0.7Aab	2.0±0.1Aa	1.7±0.58Bb
	100%	5.9±2.4Ba	1.9±0.2Aa	2.8±1.4ABa	4.3±1.95Bab
	UNIJ				
	0%	42.8±23.3Aa	39.4±11.2Ab	53.3±8.7Ab	49.8±16.0Aa
	50%	12.2±3.5Aa	23.6±7.8ABab	34.1±24.0ABab	46.4±10.4Ba
	100%	41.1±27.6Aa	16.6±5.2Aa	13.0±7.9Aa	84.8±12.2Bb
	HAB				
	0%		48.1±14.4Ab	63.6±23.0Ab	49.0±6.4Aab
	50%		24.5±12.1Aa	21.0±2.8Aa	42.0±6.4Ba
	100%		14.8±1.7Aa	25.3±3.3Aa	63.8±12.6Bb
HDCs					
	HET				
	0%	27.4±01.9ABb	23.1±4.8Ab	29.4±5.9ABb	37.0±6.0Bb
	50%	19.0±3.0Aa	15.0±5.2Aab	20.9±4.2Aab	14.5±3.1Aa
	100%	18.5±1.9BCa	10.4±2.1Aa	13.1±4.1ABa	21.8±4.1Ca
	UNIK				
	0%	17.0±3.1Bb	7.9±0.9Aa	8.9±1.4Aa	28.2±6.4Cb
	50%	11.7±2.8ABa	8.1±1.4Aa	7.5±2.7Aa	15.5±4.8Ba
	100%	14.3±1.4Bab	6.3±1.2Aa	7.7±3.0Aa	20.1±3.2Cab
	UNIJ				
	0%	73.5±10.9Ba	48.1±5.9Ab	75.2±10.5Bb	102.3±17.0Cb
	50%	97.9±10.0Cb	42.8±5.2Ab	49.8±6.4Aa	76.0±14.7Bab
	100%	84.0±14.6Bab	31.5±5.7Aa	42.8±1.7Aa	75.2±10.5Ba
	HAB				
	0%		46.3±6.3Ab	65.6±7.2Bb	71.7±2.0Bb
	50%		33.2±3.5ABa	29.7±4.9Aa	40.5±6.1Ba
	100%		27.1±1.7Aa	33.1±3.5Aa	34.9±3.5Ba

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lowercase represents WS according to Tukey's HSD post hoc test; ND: not detected.

Appendix 5: Effect of water supply on capsaicinoid concentration in the various pepper cultivars for the 2020 growing season. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Capsaicinoid	WST/cultivar	1st harvest	2nd harvest	3rd harvest
NNDC				
	HET			
	0%	nd	1.78 ± 0.09 Ba	2.12 ± 0.38 Ba
	50%	1.57 ± 0.35 Aa	2.16 ± 0.27 Aa	1.76 ± 0.31 Aa
	100%	1.86 ± 0.39 Ba	2.14 ± 0.11 Ca	1.64 ± 0.03 Aa
	UNIK			
	0%	0.00 ±0.00 Aa	2.01±0.21 Bb	nd
	50%	0.88 ±0.70 Ab	1.29±0.27 Aab	1.20 ± 0.09 Ab
	100%	1.37 ±0.70 Ab	0.84±0.13 Aa	0.88 ± 0.20 Aa
	UNIJ			
	0%	12.24 ±1.81 Ba	6.82±0.13 Aa	9.90±0.58 Bb
	50%	14.07 ±1.56 Ba	7.76±1.65 Aa	9.37±0.79 Ab
	100%	11.26 ±1.65 Ba	5.71±1.94 Aa	7.26±0.96 Aa
	HAB			
	0%	0.00 ±0.00 Aa	22.40±2.51 Bab	21.62±3.88 Bb
	50%	0.00 ±0.00 Aa	24.80±2.51 Cb	15.64±0.79 Ba
	100%	5.26± 1.30 Ab	17.80±0.36 Ca	12.35±0.09 Ba
NCAP				
	HET			
	0%	nd	0.63±0.11 Ba	0.69±0.08 Ba
	50%	0.64 ±0.11 Aa	0.70±0.07 Aa	0.62±0.08 Aa
	100%	0.52 ±0.02 Aa	0.66±0.08 ABa	0.69±0.05 Ba
	UNIK			
	0%	0.00 ±0.00 Aa	0.53±0.06 Bb	0.00±0.00 Aa
	50%	0.33 ±0.07 Ab	0.38±0.05 Aab	0.92±0.13 Bb
	100%	0.55 ±0.10 Ac	0.35±0.08 Aa	0.43±0.35 Aab
	UNIJ			
	0%	8.55 ±1.51 Ba	5.90±0.60 Aa	4.74±0.21 Aa
	50%	8.74 ±1.29 Ba	5.85±0.63 Aa	5.88±0.29 Aa
	100%	9.15 ±0.79 Ca	6.15±0.64 Ba	4.40±0.29 Aa
	HAB			
	0%	0.00 ±0.00 Aa	8.05±1.03 Ca	4.81±0.90 Ba
	50%	0.00 ±0.00 Aa	7.13±2.56 Ba	7.02±0.43 Bb
	100%	4.99 ±1.24 Ab	9.03±0.75 Ba	7.34±0.77 ABb
NDC				
	HET			
	0%	33.05 ±2.48 Aa	32.49±1.83 Aa	39.14±2.33 Bb
	50%	38.43 ±12.22 ABa	44.48±3.23 Bb	24.39±0.96 Aa
	100%	27.76 ±6.17 Aa	28.87±2.74 Aa	28.70±4.10 Aa
	UNIK			
	0%	17.33 ±6.45 Aa	32.86±0.39 Ba	21.39±4.08 Aa
	50%	13.00 ±3.55 Aa	21.58±7.45 Aa	25.30±2.60 Aa
	100%	18.69 ±4.40 Aa	22.95±6.65 Aa	18.73±4.06 Aa
	UNIJ			

	0%	48.06 ±6.00 Aa	116.26±7.07 Ba	176.14±12.93 Cb
	50%	43.49 ±2.36 Aa	136.95±44.71 Ba	150.53±5.61 Bab
	100%	41.82 ±6.65 Aa	97.60±13.75 Ba	132.43±25.02 Ba
	HAB			
	0%	57.21 ±6.39 Aa	64.59±10.85 Aab	54.61±4.27 Aab
	50%	68.14 ±10.58 Aab	77.36±2.91 Ab	69.86±9.42 Ab
	100%	79.44 ±7.79 Bb	52.18±4.53 Aa	49.33±4.05 Aa
CAP				
	HET			
	0%	274.96 ±9.78 Aa	367.20±32.33 Bab	329.27±50.30 Aa
	50%	356.94 ±46.47 ABb	455.26±61.27 Bb	259.45±26.88 Aa
	100%	299.97 ±27.16 Aab	332.53±1.48 Aa	324.07±10.92 Aa
	UNIK			
	0%	133.44 ±33.20 Aa	260.24±22.78 Bb	212.32±32.64 Bb
	50%	108.86 ±15.02 Aa	150.95±36.03 ABa	173.85±2.20 Bab
	100%	108.66 ±9.51 Aa	135.15±9.54 Aa	136.05±21.08 Aa
	UNIJ			
	0%	2841.54 ±156.40 Ba	2384.00±44.76 Ab	2206.16±118.49 Ab
	50%	2809.57 ±162.05 Ba	2179.55±66.32 Aa	1924.39±47.53 Aab
	100%	2875.77 ±411.00 Ba	2176.51±88.65 Aa	1756.51±185.74 Aa
	HAB			
	0%	1736.00 ±43.99 Aa	2695.02±10.43 Ba	2235.48±360.48 ABa
	50%	2032.15 ±185.59 Aa	3257.22±272.51 Ba	2845.36±168.22 Ba
	100%	1920.51 ±163.04 Aa	2918.60±318.12 Ba	2541.83±145.69 Ba
DC				
	HET			
	0%	188.27 ±2.65 Aa	274.99±42.67 Bb	217.75±21.75 ABb
	50%	244.96 ±50.07 Ba	217.58±8.39 ABab	159.55±22.52 Ba
	100%	186.05 ±32.67 Aa	185.27±5.05 Aa	172.39±11.71 Aab
	UNIK			
	0%	96.40 ±26.65 Aa	179.66±15.18 Bb	119.55±5.43 Aa
	50%	74.49 ±11.43 Aa	93.84±9.12 Aa	126.42±5.48 Ba
	100%	82.90 ±7.35 Aa	95.87±3.86 Aa	98.22±21.38 Aa
	UNIJ			
	0%	947.93 ±51.57 Aa	1204.45±48.79 Ba	1341.31±135.23 Bb
	50%	863.81 ±43.67 Aa	1168.09±263.72 Aa	1178.30±51.24 Aab
	100%	887.61 ±91.79 Aa	938.72±73.27 Aa	1014.35±153.05 Aa
	HAB			
	0%	925.08 ±234.25 Aa	1099.86±38.21 Aab	1242.30±122.27 Ab
	50%	883.96 ±85.66 Aa	1162.26±39.70 Bb	918.36±118.12 Aa
	100%	895.45 ±41.25 Aa	991.56±79.19 Aa	860.03±35.00 Aa
iDC				
	HET			
	0%	0.92 ±0.07 Aa	1.31±0.37 Ba	0.18±0.06 Aa
	50%	0.97 ±0.05 Ba	1.28±0.23 Ba	0.18±0.04 Aa
	100%	0.90 ±0.05 Ba	0.83±0.11 Ba	0.26±0.07 Aa
	UNIK			
	0%	1.38 ±0.80 Ba	2.07±0.06 Bb	0.00±0.00 A
	50%	1.22 ±0.37 Ba	1.31±0.34 Ba	0.00±0.00 A

	100%	1.32 ±0.13 Ba	1.98±0.33 Bab	0.00±0.00 A
	UNIJ			
	0%	2.17 ±0.54 a	0.00±0.00	0.00±0.00 A
	50%	2.18 ±1.10 Ba	5.17±0.75C	0.00±0.00 A
	100%	2.59 ±0.66 Ba	4.87±0.79C	0.00±0.00A
	HAB			
	0%	1.27 ±0.28 Ba	1.28±0.30B	0.00±0.00 A
	50%	0.76 ±0.11 Ba	2.15±0.18C	0.00±0.00 A
	100%	3.18 ±2.72 a	0.00±0.00	0.00±0.00
HCAP				
	HET			
	0%	6.77 ±1.35 Ba	4.96±0.08 ABa	3.78±0.14 Aab
	50%	5.59 ±2.08 Aa	7.25±3.78 Aa	5.06±0.98 Ab
	100%	3.94 ±0.83 ABa	4.98±0.80 Ba	2.29±0.38 Aa
	UNIK			
	0%	2.16 ±1.37 Aa	2.58±0.29 Aa	2.84±0.37 Aa
	50%	1.89 ±0.86 Aa	1.67±0.32 Aa	3.75±0.18 Bb
	100%	3.20 ±0.60 Aa	2.52±1.20 Aa	2.06±0.43 Aa
	UNIJ			
	0%	36.40 ±8.36 Ba	17.51±2.49 Aa	27.99±3.29 ABb
	50%	32.27 ±10.99 Aa	14.77±9.06 Aa	20.83±2.66 Aab
	100%	22.81 ±1.23 Ba	15.63±0.77 Aa	18.76±2.66 Ba
	HAB			
	0%	54.2 ±1.00 Aa	56.78±2.64 Ab	54.42±4.81 Aa
	50%	76.0 ±1.17 Ba	77.86±2.93 Bc	41.51±9.49 Aa
	100%	84.1 ±3.13 Ba	40.15±6.35 Aa	33.41±10.88 Aa
HDC_1				
	HET			
	0%	2.42 ±0.10 Aa	3.61±0.18 Bb	2.70±0.25 Ab
	50%	3.06 ±0.55 ABa	3.21±0.28 Bb	2.17±0.19 Aa
	100%	2.40 ±0.53 Ba	1.37±0.23 Aa	2.15±0.09 ABa
	UNIK			
	0%	0.65 ±0.31 Aa	1.05±0.08 Ab	0.65±0.06 Aa
	50%	0.61 ±0.07 Aa	0.48±0.01 Aa	0.67±0.21 Aa
	100%	0.67 ±0.14 Aa	0.91±0.28 Aab	0.64±0.24 Aa
	UNIJ			
	0%	11.95 ±1.20 Ba	8.95±0.48 Aa	9.87±0.92 ABa
	50%	11.46 ±1.08 Aa	10.86±2.37 Aa	9.43±0.67 Aa
	100%	13.21 ±1.46 Ba	8.47±1.10 Aa	8.53±0.23 Aa
	HAB			
	0%	6.83 ±0.18 Aa	18.07±2.27 Ba	16.57±1.46 Ba
	50%	7.40 ±0.63 Aa	21.25±1.07 Ba	21.08±2.37 Bb
	100%	6.74 ±0.58 Aa	17.15±3.12 Ba	13.24±0.71 Ba
HDC_2				
	HET			
	0%	14.16 ±1.01 Aa	16.06±1.95 Aa	16.90±0.69 Ab
	50%	15.32 ±4.02 ABa	22.52±6.54 Ba	11.32±0.49 Aa
	100%	12.83 ±2.41 Aa	18.93±3.55 Aa	13.44±1.45 Aa
	UNIK			

	0%	7.70 ±2.97 Aa	15.18±0.10 Bc	10.28±2.08 ABab
	50%	6.07 ±1.19 Aa	8.20±1.00 Aa	11.57±0.90 Bb
	100%	8.82 ±1.83 ABa	12.38±1.65 Bb	7.77±1.01 Aa
	UNIJ			
	0%	10.28 ±4.42 Aa	31.95±2.16 Ba	46.45±1.96 Cb
	50%	11.31 ±1.50 Aa	40.46±2.92 Bb	35.60±2.64 Ba
	100%	11.64 ±2.02 Aa	33.21±2.84 Ba	42.41±3.06 Cb
	HAB			
	0%	19.37 ±2.46 Bab	16.07±2.33 ABab	12.10±1.33 Aa
	50%	22.73 ±1.20 Bb	20.13±1.46 ABb	17.00±1.68 Ab
	100%	17.44 ±2.22 Ba	13.65±0.77 Aa	11.53±0.02 Aa

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents water supply according to Tukey's HSD post hoc test.

Appendix 6: Effect of harvesting periods and WS treatments on the concentration of the individual carotenoid groups in the 2019 season. The means are expressed in µg/g fresh base weight ± S.D (n=4).

Carotenoid	WST/cultivar	1st harvest	2nd harvest	3rd harvest	4th harvest
Free caps					
	HET				
	0%	7.7±2.6Aa	57.5±12.1Bb	39.0±15.3Ba	37.11±2.0Ba
	50%	7.4±0.8Aa	33.9±5.9Ba	29.5±7.7Ba	37.9±7.6Ba
	100%	6.3±1.8Aa	37.7±11.6BCab	19.7±12.0ABa	56.0±17.6Ca
	UNIK				
	0%	13.0±4.0Ab	27.0±3.7ABa	31.0±11.5Ba	38.7±10.8Ba
	50%	9.3±2.2Aab	52.0±7.6Cb	26.4±7.7Ba	82.9±10.4Db
	100%	6.1±1.9Aa	56.6±16.1Bb	21.5±4.0Aa	79.0±4.1Cb
	UNIJ				
	0%	4.61±2.6Aa	46.9±5.5Ba	31.2±13.4Ba	33.9±16.0Ba
	50%	3.9±1.4Aa	43.5±4.6Ba	23.4±4.2ABa	59.0±35.1Ba
	100%	1.2±1.4Aa	39.0±8.2Ca	19.2±2.8Ba	31.0±7.7BCa
	HAB				
	0%	3.9±1.8Aab	13.8±3.0Ca	8.6±2.8Ba	
	50%	2.2±1.6Aa	9.4±4.4Ba	11.3±1.1Ba	
	100%	5.3±0.8ABb	9.3±1.5BCa	13.2±5.1Ca	
Zeax ME					
	HET				
	0%	2.4±1.7Aa	55.9±15.8Ba	12.1±4.2Aa	18.7±0.9Aa
	50%	2.8±1.0Aa	30.1±12.6Ca	11.2±4.2ABa	18.6±1.1BCa
	100%	1.3±0.7Aa	40.5±11.6Ca	9.7±1.7ABa	18.0±1.8Ba
	UNIK				
	0%	5.0±4.1Aa	6.4±3.4Aa	16.4±3.1Ba	19.0±5.5Ba
	50%	4.9±1.9Aa	5.4±0.8Aa	10.4±5.0Aa	18.2±4.2Ba
	100%	3.0±1.0Aa	7.2±2.4Aa	9.1±2.8Aa	18.8±5.7Ba
	UNIJ				
	0%	3.9±0.4Aa	8.6±0.9Ab	19.9±14.6ABa	30.8±8.0Ba
	50%	6.4±1.9Aa	6.3±0.6Aa	13.8±1.8ABa	18.9±11.1Ba
	100%	5.2±3.1Aa	5.3±0.9Aa	11.8±2.1Ba	14.8±3.1Ba
	HAB				
	0%	0.7±0.3Aa	9.4±6.4Ba	5.6±1.2ABa	
	50%	0.7±0.3Aa	5.6±2.0Ba	8.4±1.2Ca	
	100%	0.5±0.1Aa	5.1±0.6Ba	6.1±1.9Ba	
β-carotene					
	HET				
	0%	2.4±1.1Ab	33.6±9.9Ca	30.3±11.3BCa	16.8±1.7ABa
	50%	2.3±0.6Ab	19.1±6.8BCa	13.1±3.8Bb	24.1±5.1Ca
	100%	0.2±0.1Aa	21.3±10.2Ba	15.6±7.2ABab	20.6±8.0Ba
	UNIK				
	0%	1.9±1.1Ab	13.8±1.7Ba	13.6±2.8Ba	18.6±7.4Bb
	50%	0.8±0.5Aab	12.1±1.1Ba	10.8±3.9Ba	4.0±0.2Aa
	100%	0.4±0.3Aa	14.5±7.3Ca	10.1±2.8BCa	4.9±0.4ABa
	UNIJ				
	0%	1.9±0.5Aa	15.6±3.9Ba	18±7.1Bb	10.5±5.6ABb

	50%	2.9±0.7Aa	14.3±5.1Ba	7.1±5.5ABa	0.3±0.2Aa
	100%	2.4±1.2Aa	13.9±6.2Ba	6.8±1.1Aa	0.3±0.1Aa
	HAB				
	0%	0.7±0.4Aa	0.9±0.8Aa	3.1±0.9Bb	
	50%	1.0±0.4Aa	0.7±0.1Aa	1.8±0.3Ba	
	100%	0.7±0.2Aa	0.6±0.1Aa	1.0±0.4Aa	
Caps DE					
	HET				
	0%	7.1±3.8Ab	133.4±25.7Bb	136.8±21.8Ba	26.7±17.6Aa
	50%	3.9±1.4Bab	80.0±19.8Bab	134.9±23.8Ca	31.3±10.6Aa
	100%	2.0±0.9Aa	95.5±24.8Ca	120.3±13.1Ca	49.2±3.4Ba
	UNIK				
	0%	6.4±3.4Ab	69.9±21.7Ba	137.6±12.2Cb	16.9±12.5Aa
	50%	3.0±2.2Aab	71.8±11.8Ba	114.9±30.0Cab	14.3±4.0Aa
	100%	1.0±0.5Aa	75.2±17.1Ca	85.0±7.2Ca	20.8±1.3Ba
	UNIJ				
	0%	23.1±2.5Ab	97.8±15.6Bb	103.5±20.1Bb	33.6±1.9Aa
	50%	18.5±5.2Aab	23.2±9.8Aa	71.2±9.4Ba	28.6±17.6Aa
	100%	13.3±3.3Aa	82.9±16.9Cb	60.2±10.7Ba	27.1±4.8Aa
	HAB				
	0%	3.1±0.9Aa	12.8±1.4Bb	35.5±8.6Ca	
	50%	9.3±3.2Ab	8.2±2.5Aa	42.7±5.0Ba	
	100%	7.8±1.3Ab	8.2±0.9Aa	30.6±10.6Ba	
Zeax DE					
	HET				
	0%	0.1±0.1Aa	3.5±1.0Aa	0.1±0.1Aa	5.3±6.2Aa
	50%	0.4±0.0Ab	1.8±1.3Aa	0.4±0.3Aa	5.9±2.6Ba
	100%	0.1±0.1Aa	2.5±0.8Ba	4.5±1.2Cb	9.8±0.7Da
	UNIK				
	0%	2.8±4.9ABa	1.6±1.6ABa	6.0±1.3Bb	ND
	50%	0.3±0.1ABa	2.7±0.5ABa	0.2±0.1Aa	2.0±1.6BCb
	100%	0.2±0.1Aa	2.3±0.7Aa	0.2±0.0Aa	ND
	UNIJ				
	0%	ND	6±2.1Ab	0.1±0.1Aa	5.7±6.6Aa
	50%	0.1±0.1Aa	0.0±0.1Aa	8.4±0.8Bb	6.6±4.1Ba
	100%	0.1±0.0Aa	0.1±0.0Aa	5.3±3.7Bb	6.6±1.6Ba
	HAB				
	0%	0.5±0.2Ab	1.9±0.3Bb	1.4±1.0ABb	
	50%	0.3±0.1Aab	1.3±0.4Ba	1.4±0.3Bb	
	100%	0.2±0.1Aa	1.3±0.3Bab	tr	

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lowercase represents WS according to Tukey's HSD post hoc test; ND: not detected.

Appendix 7: Effect of harvesting periods and water supply treatments on carotenoid concentration in the 2020 season. The means are expressed in µg/g fresh base weight ± S.D (n = 4)

Carotenoid	WST/ Cultivar	1st harvest	2nd harvest	3rd harvest
FRX				
	HET			
	0%	79.25 ± 1.09 Cb	57.10 ±1.46 Bb	32.12 ± 3.48 Aa
	50%	46.87 ± 1.36 Ba	38.90 ±7.25 ABa	31.38 ± 2.31 Aa
	100%	56.30 ± 6.32 Ca	44.14 ±1.86 Ba	30.79 ± 4.16 Aa
	UNIK			
	0%	43.38 ± 3.20 Aa	62.90 ±6.06 Ba	51.18 ± 5.17 ABb
	50%	61.64 ± 4.23 Ba	58.31 ±3.11 Ba	38.50 ± 3.11 Aa
	100%	62.73 ± 17.09 Ba	71.35 ±5.55 Bb	29.17 ± 4.70 Aa
	UNIJ			
	0%	50.33 ± 5.33 Bb	38.76 ±3.37 Ab	42.17 ± 2.10 ABa
	50%	38.87 ± 6.10 Aab	36.37 ±2.01 Ab	38.39 ± 1.23 Aa
	100%	31.72 ± 4.62 ABa	28.53 ±1.19 Aa	39.45 ± 2.81 Ba
	HAB			
	0%	1.83 ± 0.80 Aa	11.77 ±6.14 Ba	9.39 ± 1.42 ABa
	50%	7.75 ± 1.07 Ab	6.89 ±1.28 Aa	9.48 ± 0.66 Aa
	100%	11.53 ± 1.71 Ac	8.42 ±0.79 Aa	10.95 ± 3.90 Aa
ME_RX				
	HET			
	0%	98.35 ± 4.58 Ba	127.12 ±4.91 Cb	16.94 ± 1.94 Aa
	50%	92.34 ± 5.52 Ba	84.26 ±9.90 Ba	21.26 ± 2.39 Aa
	100%	88.70 ± 13.79 Ba	85.76 ±6.89 Ba	21.47 ± 2.45 Aa
	UNIK			
	0%	103.10 ± 17.32 Bb	105.09 ±1.74 Ba	27.17 ± 6.21 Aa
	50%	72.92 ± 6.04 Ba	101.95 ±7.85 Ca	22.58 ± 2.09 Aa
	100%	91.60 ± 7.94 Bab	95.56 ±8.11 Ba	18.53 ± 2.20 Aa
	UNIJ			
	0%	74.78 ± 4.01 Bb	78.24 ±8.26 Ba	37.41 ± 2.56 Ab
	50%	71.36 ± 8.01 Bb	86.33 ±4.24 Cab	23.25 ± 1.94 Aa
	100%	49.07 ± 5.58 Ba	92.65 ±2.41 Cb	25.89 ± 0.44 Aa
	HAB			
	0%	15.64 ± 2.39 Aa	40.24 ±7.57 Ba	11.24 ± 3.78 Aa
	50%	22.91 ± 4.21 Ba	40.28 ±4.53 Ca	12.69 ± 2.07 Aa
	100%	30.87 ± 10.63B a	51.56 ±2.54 Ca	12.78 ± 1.81 Aa
DE_RX				
	HET			
	0%	243.69 ± 10.37 Ba	384.71 ±27.05 Cb	103.19 ± 9.84 Aa
	50%	268.05 ± 2.84 Bb	229.51 ±37.02 Ba	97.88 ± 8.82 Aa
	100%	288.82 ± 8.52 Bc	256.49 ±20.95 Ba	99.91 ± 5.53 Aa
	UNIK			
	0%	342.10 ± 28.36 Cb	218.06 ±20.11 Bb	87.34 ± 14.10 Ab
	50%	189.12 ± 15.71 Ca	153.30 ±15.74 Ba	72.15 ± 3.42 Aab
	100%	237.34 ± 22.61 Ca	180.94 ±16.76 Bab	56.05 ± 9.16 Aa

	UNIJ			
	0%	215.45 ± 5.67 Ca	161.76 ±12.94 Ba	75.86 ± 9.33 Aa
	50%	225.63 ± 24.98 Ba	168.37 ±70.32 ABa	69.38 ± 8.38 Aa
	100%	191.67 ± 8.54 Ba	201.70 ±13.39 Ba	74.43 ± 13.48 Aa
	HAB			
	0%	77.65 ± 8.42 Ba	110.50 ±9.89 Ca	35.50 ± 5.71 Aa
	50%	120.48 ± 4.96 Bb	122.26 ±10.35 Bab	36.72 ± 4.60 Aa
	100%	116.75 ± 9.56 ABb	178.44 ±43.42 Bb	52.74 ± 3.00 Ab
FYX				
	HET			
	0%	64.20 ± 3.54 Bb	39.92 ±2.92 Ab	43.35 ± 4.75 Aa
	50%	36.98 ± 1.36 Ba	28.16 ±3.36 Aa	40.01 ± 1.41 Ba
	100%	33.40 ± 8.71 Aa	27.99 ±2.28 Aa	39.70 ± 2.12 Aa
	UNIK			
	0%	33.62 ± 2.89 Aa	35.21 ±2.88 Aa	46.70 ± 5.99 Bb
	50%	42.26 ± 2.03 Bb	34.42 ±3.88 Aa	49.60 ± 2.37 Cb
	100%	41.09 ± 3.19 Bb	38.50 ±2.64 Ba	27.43 ± 5.64 Aa
	UNIJ			
	0%	30.15 ± 1.56 Ab	35.50 ±4.56 ABa	39.32 ± 1.31 Ab
	50%	26.32 ± 4.21 Aab	36.59 ±2.92 Ba	25.06 ± 1.42 Aa
	100%	22.29 ± 1.09 Aa	32.99 ±7.60 Aa	29.41 ± 2.80 Aa
	HAB			
	0%	5.16 ± 0.20 Aa	11.67 ±2.45 Ba	17.58 ± 2.07 Ca
	50%	7.11 ± 1.33 Aab	11.76 ±2.99 Aa	25.90 ± 3.40 Bb
	100%	8.04 ± 0.65 Ab	14.60 ±1.67 Ba	30.33 ± 0.37 Cb
ME-Y xanthophylls				
	HET			
	0%	97.97 ± 2.63 Ab	168.55 ±13.06 Cb	132.42 ± 7.33 Bb
	50%	103.43 ± 6.54 Ab	104.98 ±14.47 Aa	107.15 ± 5.70 Aa
	100%	76.05 ± 7.79 Aa	105.22 ±10.79 Ba	110.35 ± 5.05 Ba
	UNIK			
	0%	126.84 ± 11.26 Bb	88.80 ±5.85 Aa	110.96 ± 13.21 ABb
	50%	98.96 ± 9.23 Ba	71.00 ±7.96 Aa	77.91 ± 1.52 Aa
	100%	112.20 ± 10.51 Bab	68.69 ±10.12 Aa	70.01 ± 7.37 Aa
	UNIJ			
	0%	98.90 ± 6.98 Ba	81.42 ±4.20 Aa	93.64 ± 6.27 Ba
	50%	85.70 ± 12.89 Ba	106.47 ±16.34 Aa	92.40 ± 22.26 Aa
	100%	79.98 ± 5.40 Ba	90.28 ±7.17 Aa	108.81 ± 15.93 Ba
	HAB			
	0%	35.52 ± 3.90 Aa	40.83 ±8.13 Aa	57.57 ± 4.42 Ba
	50%	52.04 ± 3.64 ABb	49.30 ±4.74 Aab	62.72 ± 5.45 Ba
	100%	55.49 ± 3.53 Ab	54.78 ±0.42 Ab	102.74 ± 9.44 Bb
DE-Y xanthophylls				
	HET			
	0%	25.46 ± 1.75 Bb	39.03 ±5.15 Cb	14.75 ± 0.50 Aa
	50%	18.67 ± 1.81 Aa	18.14 ±3.77 Aa	14.75 ± 1.58 Aa
	100%	22.37 ± 1.08 Aab	30.84 ±4.97 Bb	18.53 ± 1.26 Ab
	UNIK			

	0%	21.55 ± 3.49 Aa	30.60 ±3.31 Bb	15.86 ± 0.80 Ab
	50%	28.72 ± 1.43 Cab	23.65 ±2.12 Ba	10.99 ± 0.94 Aa
	100%	30.99 ± 4.80 Bb	27.03 ±1.70 Bab	8.88 ± 1.57 Aa
	UNIJ			
	0%	39.35 ± 4.43 Ba	16.34 ±1.15 Aa	48.12 ± 3.07 Cb
	50%	31.56 ± 4.71 Aa	21.10 ±6.75 Aa	33.65 ± 3.24 Aa
	100%	34.45 ± 4.88 Ba	21.44 ±6.82 Aa	42.90 ± 1.81 Bb
	HAB			
	0%	7.81 ± 0.46 Aa	7.55 ±1.01 Aa	9.60 ± 1.63 Aa
	50%	11.27 ± 0.75 Bb	10.27 ±1.10 ABab	8.48 ± 0.11 Aa
	100%	5.98 ± 1.79 Aa	11.50 ±2.12 Bb	14.45 ± 1.29 Bb
Total Red				
	HET			
	0%	421.30 ± 13.94 Ba	568.93 ±29.00 Cb	267.73 ± 20.50 Aa
	50%	407.26 ± 6.32 Ba	352.68 ±44.79 Ba	236.41 ± 15.70 Aa
	100%	433.82 ± 26.10 Ba	386.39 ±28.20 Ba	241.05 ± 11.72 Aa
	UNIK			
	0%	488.58 ± 42.86 Bb	386.04 ±22.10 Bb	212.49 ± 79.37 Aa
	50%	320.35 ± 30.16 Ba	313.57 ±24.74 Ba	188.56 ± 3.73 Aa
	100%	391.67 ± 42.48 Bab	347.85 ±22.39 Bab	155.23 ± 20.43 Aa
	UNIJ			
	0%	340.56 ± 11.52 Cb	278.75 ±23.78 Ba	211.66 ± 16.46 Aa
	50%	335.85 ± 32.85 Bb	291.07±69.59ABa	200.17 ± 20.50 Aa
	100%	272.46 ± 6.37 ABa	322.87 ±12.35 Ba	226.03 ± 37.72 Aa
	HAB			
	0%	95.12 ± 10.56 Aa	162.51 ±22.65 Ca	102.46 ± 11.40 Ba
	50%	151.15 ± 8.35 Ab	169.43 ±16.03 Bab	108.92 ± 10.68 Ba
	100%	159.15 ± 19.26 Ab	238.42 ±42.40 Bb	166.44 ± 15.92 Ab
TotalYellow				
	HET			
	0%	255.98 ± 8.26 Bb	314.67 ±24.70 Cb	156.53 ± 13.32 Aa
	50%	205.27 ± 15.87 Ba	203.07 ±20.34 Ba	144.01 ± 4.60 Aa
	100%	177.09 ± 21.64 ABa	215.55 ±21.72 Ba	155.14 ± 13.81 Aa
	UNIK			
	0%	234.11 ± 16.59 Ba	235.59 ±15.15 Bb	166.72 ± 22.14 Ab
	50%	213.13 ± 18.50 Ba	188.55 ±5.69 Ba	139.82 ± 6.08 Aab
	100%	222.61 ± 16.46 Ba	205.13 ±13.29 Ba	100.27 ± 15.64 Aa
	UNIJ			
	0%	218.07 ± 10.60 Aa	224.33 ±11.35 Aa	225.21 ± 6.47 Ac
	50%	180.69 ± 30.67 Aa	254.34 ±38.79 Ba	158.57 ± 10.22 Aa
	100%	174.49 ± 8.29 Aa	226.09 ±7.45 Ba	186.77 ± 5.93 Aa
	HAB			
	0%	68.54 ± 5.23 Aa	137.79 ±15.27 Ca	103.22 ± 17.22 Ba
	50%	103.81 ± 7.00 Ab	148.49 ±12.03 Ba	122.03 ± 18.91 Aa
	100%	103.14 ± 9.23 Ab	161.09 ±4.17 Ba	177.12 ± 5.15 Bb
R/Y				
	HET			
	0%	1.65 ± 0.07 Aa	1.81 ±0.05 Ba	1.71 ± 0.03 ABb
	50%	1.99 ± 0.12 Bb	1.73 ±0.05 Aa	1.64 ± 0.07 Aab
	100%	2.46 ± 0.15 Bc	1.80 ±0.05 Aa	1.56 ± 0.06 Aa

	UNIK			
	0%	2.09 ± 0.05 Bc	1.64 ±0.08 ABa	1.25 ± 0.36 Aa
	50%	1.50 ± 0.04 Ba	1.66 ±0.09 Ca	1.35 ± 0.04 Aa
	100%	1.76 ± 0.07 Bb	1.70 ±0.06 ABa	1.55 ± 0.04 Aa
	UNIJ			
	0%	1.56 ± 0.07 Ca	1.24 ±0.06 Bab	0.94 ± 0.10 Aa
	50%	1.88 ± 0.21 Ba	1.14 ±0.14 Aa	1.26 ± 0.14 Aa
	100%	1.56 ± 0.04 Ba	1.43 ±0.01 ABb	1.21 ± 0.17 Aa
	HAB			
	0%	1.39 ± 0.06 Ca	1.18 ±0.04 Ba	1.00 ± 0.09 Aa
	50%	1.46 ± 0.02 Ca	1.14 ±0.08 Ba	0.90 ± 0.09 Aa
	100%	1.54 ± 0.16 Ba	1.48 ±0.22 Ba	0.94 ± 0.12 Aa
Sum L/Z				
	HET			
	0%	77.78 ± 7.25 Bb	71.13 ±8.66 Bb	52.17 ± 3.65 Ab
	50%	42.43 ± 2.65 Aa	40.90 ±4.53 Aa	43.70 ± 1.93 Aa
	100%	41.13 ± 4.01 Aa	43.16 ±7.00 ABa	56.28 ± 5.77 Bab
	UNIK			
	0%	26.35 ± 3.69 Aa	88.80 ±9.42 Ba	33.17 ± 4.61 Ab
	50%	45.75 ± 1.95 Bb	72.45 ±7.18 Ca	25.06 ± 0.99 Aa
	100%	43.24 ± 2.28 Bb	75.20 ±6.37 Ca	18.92 ± 2.30 Aa
	UNIJ			
	0%	38.15 ± 3.60 Aa	49.51 ±4.63 Ba	59.17 ± 2.07 Cb
	50%	34.20 ± 3.93 Aa	61.69 ±9.35 Ba	46.07 ± 3.77 ABa
	100%	40.23 ± 3.89 Aa	56.87 ±9.71 Ba	52.66 ±2.28 ABab
	HAB			
	0%	6.55 ± 0.39 Aab	25.47 ±4.09 Ca	16.06 ± 1.97 Ba
	50%	5.78 ± 0.58 Aa	27.70 ±3.07 Ca	15.94 ± 1.22 Ba
	100%	7.51 ± 0.75 Ab	32.95 ±3.50 Ca	20.15 ± 1.53 Bb
Sum β- cryptxanthin				
	HET			
	0%	11.27 ± 1.47 Ba	18.68 ±1.64 Cb	7.12 ± 0.50 Aa
	50%	10.13 ± 2.56 Aa	10.46 ±2.46 Aa	9.11 ± 0.77 Ab
	100%	20.32 ± 1.34 Bb	17.48 ±1.71 Bb	6.05 ± 0.95 Aa
	UNIK			
	0%	23.56 ± 2.88 Ba	14.97 ±2.33 Ab	9.81 ± 1.05 Ab
	50%	20.15 ± 0.78 Ba	7.51 ±0.14 Aa	6.76 ± 1.32 Aa
	100%	19.62 ± 1.40 Ca	13.12 ±0.52 Bb	4.91 ± 0.92 Aa
	UNIJ			
	0%	18.56 ± 1.71 Ab	21.55 ±1.54 Ba	11.36 ± 1.22 Ab
	50%	13.56 ± 1.86 ABa	27.59 ±11.49 Ba	6.96 ± 1.92 Aa
	100%	14.98 ± 1.00 Bab	21.30 ±2.66 Ca	10.00 ± 1.94 Aab
	HAB			
	0%	6.62 ± 0.35 Aa	7.99 ±1.59 Aa	7.87 ± 1.26 Aa
	50%	9.91 ± 1.85 Ab	10.94 ±0.70 Ab	9.62 ± 0.76 Aa
	100%	11.41 ± 0.33 Ab	15.65 ±0.76 Bc	13.32 ± 1.51 ABb
β carotene				
	HET			
	0%	33.62 ± 1.71 Aa	65.38 ±4.12 Bb	68.34 ± 5.37 Ba
	50%	45.65 ± 6.60 Aa	50.25 ±2.96 ABa	59.54 ± 1.70 Ba

	100%	44.69 ± 5.23 Aa	49.67 ±4.35 Aa	63.49 ± 6.13 Ba
	UNIK			
	0%	51.22 ± 2.23 Ab	64.52 ±2.13 Bc	57.64 ± 8.48 ABb
	50%	39.93 ± 7.37 Aa	45.86 ±3.23 Aa	43.65 ± 4.04 Aab
	100%	35.15 ± 0.71 Aa	57.92 ±1.81 Bb	39.21 ± 6.59 Aa
	UNIJ			
	0%	47.59 ± 2.94 Aa	75.83 ±7.27 Ba	67.86 ± 5.93 Bb
	50%	35.91 ± 8.83 Aa	72.52 ±12.68 Ba	47.57 ± 5.18 Aa
	100%	36.11 ± 4.02 Aa	64.47 ±4.99 Ba	55.27 ± 2.77 Ba
	HAB			
	0%	19.74 ± 1.19 Aa	67.34 ±3.82 Ba	53.17 ± 10.14 Ba
	50%	32.91 ± 4.13 Ab	67.08 ±5.44 Ba	63.03 ± 13.67 Ba
	100%	32.96 ± 3.88 Ab	69.71 ±0.81 Ba	104.71 ± 5.26 Cb
Cis β-carotene				
	HET			
	0%	1.09 ± 0.28 Ab	0.81 ±0.06 Aa	1.64 ± 0.10 Ba
	50%	0.39 ± 0.22 Aa	0.66 ±0.11 Aa	1.45 ± 0.15 Ba
	100%	0.58 ± 0.24 Aab	0.82 ±0.07 Aa	1.63 ± 0.13 Ba
	UNIK			
	0%	0.21 ± 0.36 Aa	1.30 ±0.23 Bb	2.33 ± 0.37 Cb
	50%	1.33 ± 0.10 Bb	0.75 ±0.25 Aa	1.77 ± 0.30 Bab
	100%	1.18 ± 0.31 Bb	0.58 ±0.15 Aa	1.50 ± 0.18 Ba
	UNIJ			
	0%	0.84 ± 0.09 Ab	0.95 ±0.16 Aa	12.26 ± 1.27 Bb
	50%	0.63 ± 0.12 Aab	2.65 ±0.56 Bb	2.09 ± 1.18 ABa
	100%	0.47 ± 0.12 Aa	2.50 ±0.30 Bb	3.60 ± 0.21 Ca
	HAB			
	0%	0.31 ± 0.13 Aa	0.38 ±0.16 Aa	5.28 ± 0.35 Ba
	50%	0.49 ± 0.15 Aab	0.33 ±0.07 Aa	5.22 ± 0.74 Ba
	100%	0.66 ± 0.12 Ab	0.44 ±0.08 Aa	8.67 ± 1.29 Bb
T vit A				
	HET			
	0%	45.97 ± 3.38 Aa	84.87 ±5.81 Bb	77.10 ± 5.92 Ba
	50%	56.16 ± 7.15 Aab	61.37 ±3.48 ABa	70.10 ± 1.64 Ba
	100%	65.59 ± 5.77 Ab	67.97 ±6.01 Aa	71.17 ± 7.13 Aa
	UNIK			
	0%	74.98 ± 2.40 Ab	80.78 ±4.31 Ac	69.79 ± 9.87 Ab
	50%	61.42 ± 8.05 Aa	54.12 ±3.23 Aa	52.18 ± 4.11 Aab
	100%	55.95 ± 1.81 Aa	71.63 ±1.30 Bb	45.62 ± 7.58 Aa
	UNIJ			
	0%	66.99 ± 4.39 Aa	98.32 ±8.66 Ba	91.47 ± 7.39 Bb
	50%	50.11 ± 10.65 Aa	102.77 ±21.80 Ba	56.62 ± 2.46 Aa
	100%	51.57 ± 2.96 Aa	88.26 ±7.26 Ca	68.87 ± 3.73 Ba
	HAB			
	0%	26.36 ± 1.37 Aa	75.71 ±5.26 Ba	66.32 ± 10.78 Ba
	50%	42.82 ± 5.87 Ab	78.34 ±6.09 Ba	77.87 ± 13.50 Ba
	100%	44.37 ± 3.56 Ab	85.79 ±0.82 Ba	126.70 ± 7.07 Cb
Total				
	HET			
	0%	668.41 ± 18.08 Ba	883.60 ±53.67 Cb	427.14 ± 33.93 Aa

50%	610.26 ± 24.23 Ba	555.75 ±65.12 Ba	382.89 ± 21.77 Aa
100%	605.36 ± 44.34 Ba	601.94 ±49.88 Ba	398.41 ± 25.42 Aa
UNIK			
0%	722.69 ± 59.16 Bb	627.83 ±35.80 Bb	410.23 ± 47.86 Ab
50%	533.48 ± 48.34 Ba	506.62 ±29.56 Ba	326.64 ± 9.02 Aab
100%	614.29 ± 58.61 Bab	558.69 ±34.94 Bab	251.13 ± 36.16 Aa
UNIJ			
0%	554.58 ± 18.63 Bb	503.90 ±34.15 Ba	428.76 ± 9.46 Ab
50%	512.76 ± 58.39 ABab	546.22 ±106.76 Ba	347.87 ± 24.16 Aa
100%	442.26 ± 13.76 Aa	550.00 ±19.84 Ba	402.97±35.04 Aab
HAB			
0%	163.98 ± 15.78 Aa	300.30 ±37.83 Ba	205.68 ± 28.01 Aa
50%	255.53 ± 15.43 Ab	317.92 ±26.00 Bab	230.95± 28.50 Aab
100%	262.87 ± 25.55 Ab	399.51 ±46.36 Bb	343.56 ± 11.36 Bb

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents water supply according to Tukey's HSD post hoc.

Appendix 8: Effect of harvest time and WS treatments on tocopherol compounds during 2020. The means are expressed in µg/g fresh weight base ± S.D (n = 4).

Tocopherol group	WST/ Cultivar	1st harvest	2nd harvest	3rd harvest
γ+β-Toc				
	HET			
	0%	0.84 ± 0.09 Ab	0.67 ± 0.15 Aa	0.91 ±0.16 Aa
	50%	0.57 ± 0.12 Aa	0.89 ± 0.13 Ba	0.70 ±0.09 ABa
	100%	0.82 ± 0.10 Bab	0.59 ± 0.17 Aa	0.82 ±0.10 Ba
	UNIK			
	0%	1.56 ± 0.93 Aa	1.41 ± 0.18 Aa	1.62 ±0.18 Aa
	50%	0.86 ± 0.27 Aa	1.47 ± 0.15 Ba	1.69 ±0.22 Ba
	100%	0.84 ± 0.09 Aa	1.65 ± 0.19 Ba	1.65 ±0.06 Ba
	UNIJ			
	0%	0.66 ± 0.04 Ba	0.21 ± 0.12 Ab	0.28 ±0.05 Ab
	50%	1.74 ± 0.50 Bb	0.29 ± 0.01 Ac	0.25 ±0.07 Aab
	100%	1.41 ± 0.34 Bab	0.12 ± 0.02 Aa	0.15 ±0.01 Aa
	HAB			
	0%	0.05 ± 0.01 Ab	0.45 ± 0.10 Cb	0.25 ±0.03 Ba
	50%	0.06 ± 0.01 Ab	0.24 ± 0.06 Ba	0.28 ±0.03 Ba
	100%	0.00 ± 0.00 Aa	0.24 ± 0.04 Ba	0.26 ±0.03 Ba
α-Toc-HQ				
	HET			
	0%	38.01 ± 3.43 Ab	38.04 ± 2.93 Ab	44.87 ±4.50 Ab
	50%	21.60 ± 5.64 Aa	35.58 ± 4.52 Bb	29.37 ±2.81 ABa
	100%	34.98 ± 3.17 Bb	18.80 ± 0.99 Aa	34.83 ±3.34 Ba
	UNIK			
	0%	29.57 ± 2.58 Aa	48.14 ±7.13 Ba	49.22 ±2.26 Ba
	50%	40.98 ± 4.89 Ab	41.95 ±5.30 ABa	54.65 ±6.03 Ba
	100%	38.01 ± 3.43 Aab	38.81 ±3.47 Aa	49.79 ±1.61 Ba
	UNIJ			
	0%	40.66 ± 1.21 Ba	9.02 ±0.89 Ab	9.42 ±0.47 Ab
	50%	32.18 ± 8.41 Ba	9.97 ±0.82 Ab	8.73 ±1.72 Aab
	100%	32.70 ± 0.80 Ba	6.01 ±0.74 Aa	6.03 ±1.15 Aa
	HAB			
	0%	1.39 ± 0.16 Aa	11.08 ±1.57 Ba	13.07 ±1.61 Ba
	50%	1.85 ± 0.58 Aab	11.08 ±1.79 Ba	13.43 ±2.01 Ba
	100%	3.53 ± 0.42 Ab	12.64 ±2.80 Ba	14.51 ±0.29 Ba
α-Toc				
	HET			
	0%	78.02 ± 3.55 Bb	75.54 ±1.53 ABb	66.74 ±4.99 Aa
	50%	67.82 ± 2.69 Aa	67.24 ±3.53 Aa	70.95 ±4.46 Aa
	100%	73.83 ± 2.67 Aab	67.82 ±2.69 Aab	73.83 ±2.67 Aa
	UNIK			
	0%	52.26 ± 4.50 Aa	78.10 ±2.99 Bb	69.50 ±8.59 Ba
	50%	81.39 ± 6.40 Bb	68.66 ±1.47 Aa	63.25 ±3.07 Aa
	100%	78.02 ± 3.55 Bb	74.95 ±4.39 Bab	57.92 ±3.04 Aa

	UNIJ			
	0%	77.72 ± 2.74 Bb	60.86 ±0.94 Aa	72.52 ±2.17 Bb
	50%	64.99 ± 6.71 Aa	66.35 ±1.03 Ab	63.24 ±4.84 Aa
	100%	64.65 ± 0.69 Aa	69.45 ±2.17 Bb	64.07 ±2.25 Aa
	HAB			
	0%	0.52 ± 0.03 Ab	36.38 ±6.90 Ba	34.70 ±2.74 Bab
	50%	0.16 ± 0.02 Aa	31.43 ±7.52 Ba	33.14 ±1.22 Ba
	100%	0.56 ± 0.24 Ab	32.97 ±2.60 Ba	39.97 ±3.50 Cb
α-Toc-Es				
	HET			
	0%	5.77 ± 1.87 Aa	5.47 ±0.90 Aa	11.58 ±1.16 Bb
	50%	5.71 ± 0.28 Aa	5.17 ±0.66 Aa	8.89 ±0.77 Ba
	100%	5.31 ± 0.59 Aa	6.08 ±0.39 Aa	12.31 ±0.59 Bb
	UNIK			
	0%	3.27 ± 0.07 Aa	6.40 ±0.29 Bb	11.48 ±0.93 Cb
	50%	5.57 ± 0.94 Aa	5.43 ±0.44 Aa	9.61 ±0.32 Ba
	100%	5.77 ± 1.87 Aa	6.66 ±0.20 Ab	8.20 ±0.48 Aa
	UNIJ			
	0%	5.80 ± 0.30 Bb	3.07 ±0.24 Aa	9.22 ±0.60 Cb
	50%	3.90 ± 0.71 Aa	3.57 ±0.13 Aa	7.62 ±0.61 Ba
	100%	4.63 ± 0.31 Bab	3.74 ±0.38 Aa	7.74 ±0.30 Ca
	HAB			
	0%	0.00 ± 0.00 Aa	1.35 ±0.28 Ba	1.76 ±0.21 Bab
	50%	0.00 ± 0.00 Aa	1.20 ±0.12 Ba	1.93 ±0.12 Cb
	100%	0.41 ± 0.07 Ab	1.18 ±0.22 Ba	1.49 ±0.16 Ca

Uppercase letters in the first data row and the lowercase letters in the first data column. The upper letter represents harvest periods, and the lower case represents WS according to Tukey's HSD post hoc test

Appendix 9: Effect of drying methods and conditions on the concentration (µg.g^{-1}dwb) of the individual capsaicinoids of peppers from different hybrids.

	Drying treatments			
Capsaicinoids	**Natural**	**Batch 60°C**	**Gradual 90-25°C**	**Before drying**
	HET			
NNDC	17.79±1.60b	18.49±5.21b	11.76±1.92a	12.68±0.64a
NCAP	5.410.53a	5.07±1.07a	3.83±0.85a	4.4±0.76a
NDC	165.00±6.04c	224.66±12.52b	198.13±36.93b	250.44±42.29a
CAPS	1929.78±192.27b	2223.26±201.57b	1870.17±245.86b	2689.65±312.67a
DC	1173.59±99.04b	1330.68±127.57b	1162.71±185.50b	1771.91±378.34a
iDC	0	3.36±0.60b	0	10.16±2.46a
Hcaps	58.99±6.54c	35.51±3.52a	28.41±1.20b	35.39±0.59a
HDC_1	15.32±2.64b	19.18±2.92b	17.19±2.19b	24.58±2.38a
HDC_2	87.97±3.69b	101.52±4.81ab	90.59±13.10ab	114.68±13.99a
	UNIK			
NNDC	9.62±2.08a	1.37±0.30c	7.42±0.89b	13.04±3.64a
NCAP	4.82±0.64b	3.08±0.11c	5.39±1.18b	3.76±0.46a
NDC	122.39±17.63b	128.02±9.83b	114.45±5.97b	234.70±2.77a
CAPS	1342.58±115.57c	928.57±98.10b	857.60±121.95b	1858.82±162.75a
DC	902.74±71.20b	655.89±68.86b	707.21±41.01b	1283.25±108.42a
iDC	0	0	0	14.81±0.41
Hcaps	26.97±10.46c	26.92±2.17c	34.48±0.57b	18.43±2.10a
HDC_1	5.611±0.48c	9.01±0.73b	8.90±2.19b	7.53±0.61a
HDC_2	78.53±5.45c	64.07±3.64b	58.83±5.13b	108.41±1.23a
	UNIJ			
NNDC	44.48±5.85b	50.61±4.11b	44.91±2.36b	56.82±1.07a
NCAP	32,07±5.05b	34.85±5.02b	33,.95±1.51b	49.19±5.04a
NDC	706.32±100.37b	698.18±31.82b	653.07±87.07b	968.85±58.89a
CAPS	14948.28±1569.49c	12361.61±726.37b	13158.03±219.38bc	19866.64±373.04a
DC	8005.78±1187.21b	6823.49±270.53b	6286.62±526.17b	10037.05±406.62a
iDC	0	0	0	0,00
Hcaps	113.89±26.24a	122.09±13.74a	122.71±20.95a	145.95±20.71a
HDC_1	62.46±±6.81a	72.53±5.20a	66.04±4.65a	74.62±4.01a
HDC_2	267.26±28.65a	242.65±6.07a	207.60±11.12b	266.24±18.04a

The same letter shows no significant difference between drying methods in the content of the individual capsaicinoids according to Tukey's HSD post hoc test (at $P<0.05$).

Appendix 10: Change in the carotenoid groups content (µg.g^{-1} dwb) as a function of natural and thermal drying of the new hybrids at different conditions.

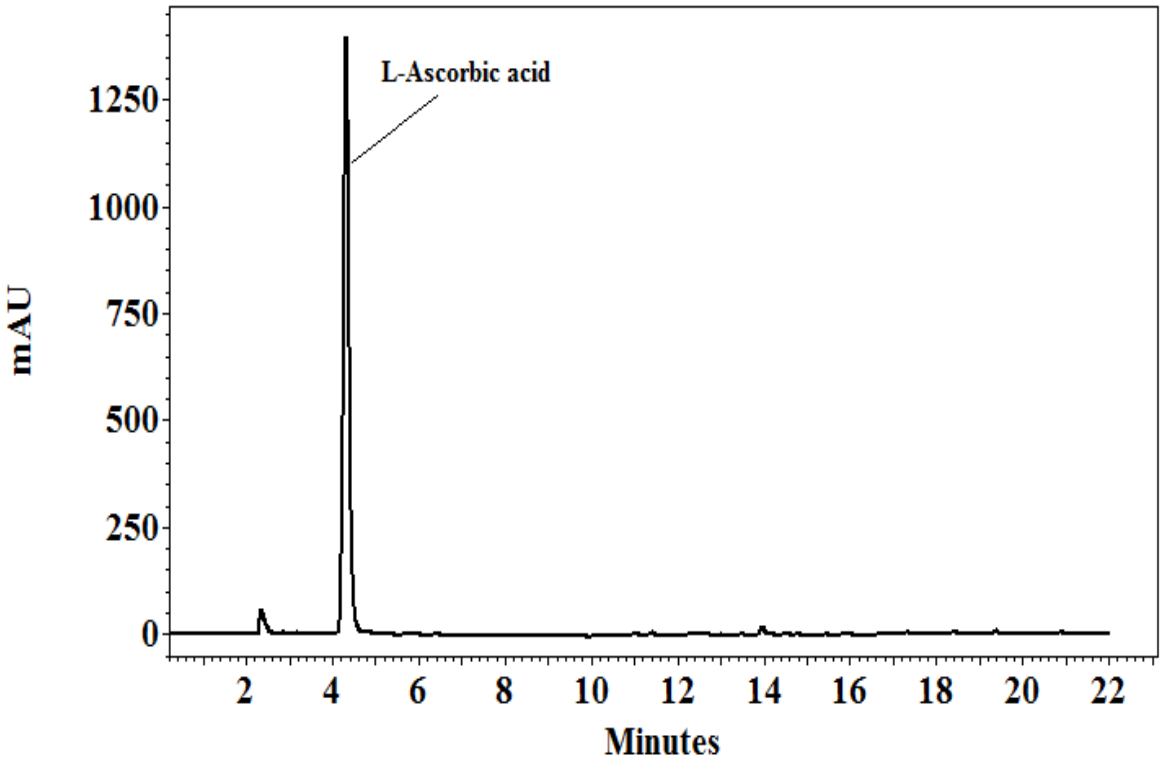
	Drying treatments			
Carotenoid groups	**Before drying**	**Natural**	**Thermal 60°C**	**Thermal 90-25°C**
		HET		
Free Red Xanthophylls	392.72±22.09a	76.72±10.17b	279.90±11.63c	263.15±14.46c
MEs of red xanthophylls	908.01±35.04a	362.15±27.76b	294.41±24.12c	382.49±67.94ca
DEs of red Xanthophylls	2725.65±170.74a	1774.11±71.52b	1245.80±82.96c	1086.59±117.03c
Free Yellow Xanthophylls	295.04±20.97a	78.29±8.6b5	179.44±5.62c	136.51±19.13d
MEs of Yellow Xanthophylls	963.99±58.37a	514.16±15.24b	418.37±28.73c	281.39±32.19d
DEs of Yellow Xanthophylls	275.88±31.74a	176.67±12.55b	99.38±8.93c	95.33±4.61c
Total Red	4026.39±163.38a	2291.29±61.13b	1999.56±110.41b	1868.76±213.76b
Total Yellow	1600.85±109.41a	1047.37±54.86b	943.14±22.07b	722.11±83.19b
Red/Yellow	2.52±0.07a	2.19±0.16b	2.12±0.14b	2.56±0.15a
Sum of Lutein + zeaxanthin	387.78±42.23a	179.39±15.73b	170.07±12.9b	146.49±10.61c
Sum of β-cryptoxanthin	133.43±14.6a	50.11±4.15b	67.46±12.42b	87.56±7.15c
β-carotene	467.02±29.46a	275.96±25.80b	239.58±10.11c	201.15±28.04c
cis β-carotenes	4.00±0.51a	2.28±0.05b	6.36±0.88c	7.57±0.93c
Total pro Vitamin A	604.46±41.60a	328.35±30.99b	313.41±23.40b	296.29±36.13b
		UNIK		
Free Red Xanthophylls	447.70±42.94a	80.38±5.93b	304.69±17.29c	257.63±23.82c
MEs of red xanthophylls	691.11±7.40a	593.99±49.25b	542.91±63.42bc	453.07±66.92c
DEs of red Xanthophylls	1375.66±164.75a	1791.72±125.01b	1289.72±147.04a	864.21±101.36c
Free Yellow Xanthophylls	316.48±13.23a	180.06±20.97b	199.88±18.89b	157.67±23.46b
MEs of Yellow Xanthophylls	765.86±87.09a	584.48±28.53b	413.47±39.79c	406.08±48.95c
DEs of Yellow Xanthophylls	345.33±31.54a	166.32±15.10b	99.38±9.03c	95.33±9.36c
Total Red	2514.47±205.22a	2466.09±180.19a	2137.33±227.75a	1574.92±192.10b
Total Yellow	1492.12±132.35a	1401.44±128.94a	958-68±78.70b	876.82±95.51b
Red/Yellow	1.68±0.02a	2.26±0.08b	2.23±0.09b	1.78±0.08a
Sum of Lutein + zeaxanthin	677.83±70.25a	330.33±28.02b	311.04±34.49b	581.62±56.98a
Sum of β-cryptoxanthin	145.76±9.72a	110.41±11.61b	61.27±9.15c	47.44±4.42c
β-carotene	452.16±26.34a	468.29±64.29a	239.58±10.11b	210.15±12.81b
cis β-carotenes	8.12±0.39a	2.23±0.35b	12.23±2.63c	12.45±1.25c
Total pro Vitamin A	606.06±36.17a	580.99±79.95a	307.21±20.14b	265.17±18.16b
		UNIJ		
Free Red Xanthophylls	245.76±49.13a	555.85±34.39b	396.64±17.75c	384.04±26.87c
MEs of red xanthophylls	555.60±71.15a	1011.87±26.08b	474.41±16.05c	438.45±20.86c
DEs of red Xanthophylls	1237.95±89.96a	2182.69±111.24b	1088.53±49.90b	1125.78±70.23a
Free Yellow Xanthophylls	209.67±29.37a	301.82±11.03b	319.15±14.43b	294.98±20.83b

MEs of Yellow Xanthophylls	617.73±16.92a	859.55±68.94b	511.11±26.33c	484.99±27.53c
DEs pf Yellow Xanthophylls	166.58±1.85a	259.13±16.00b	194.71±3.35c	156.71±7.62a
Total Red	2039.32±209.55a	3726.20±141.17b	1959.58±83.70a	1948.27±117.96a
Total Yellow	1070.50±41.30a	2215.79±127.27b	1024.97±71.11a	936.68±55.98c
Red/Yellow	1.90±0.12a	1.68±0.08b	1.91±0.05a	2.08±0.06ac
Sum of Lutein + zeaxanthin	633.02±37.55a	541.92±28.00b	457.24±46.53c	367.07±21.33d
Sum of β-cryptoxanthin	32.64±3.27a	30.00±1.06a	67.32±2.71b	23.95±2.18c
β-carotene	541.52±51.82a	765.29±30.24b	397.33±12.85c	348.68±16.01c
cis β-carotenes	4.88±0.58a	13.94±0.91b	6.79±0.91c	6.40±0.60c
Total pro Vitamin A	579.04±49.59a	934.79±40.34b	531.64±25.65a	437.02±24.22c

MEs= monoesters, DEs = di-esters.

The same letter shows no significant difference between drying methods in the content of the carotenoid groups according to Tukey's HSD post hoc test ($P<0.05$).

Appendix 11: HPLC profile of L-ascorbic acid separated on Aqua C18, 3u, 150 x 0.46 mm column with gradient elution of (B) Acetonitrile in (A) 0.01M KH_2PO_4 buffer and DAD detection at 265nm.



Tables and Figures