Hungarian University of Agriculture and Life Sciences

EFFECT OF WATER SUPPLY ON PHYSIOLOGICAL FACTORS AND RESPONSE TO GAMMA IRRADIATION OF PHYTONUTRIENTS IN CHILLI PEPPER CULTIVARS *(CAPSICUM* SP.)

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List of legend and abbreviations

SPAD: Soil Plant Analysis Development

SHU: Scoville heat units

NDC: Nordihydrocapsaicin

CAP: Capsaicin

DC: Dihydrocapsaicin

HCAP: Homocapsaicin

iDC: dihydrocapsaicin isomer

HDCs Homodihydrocapsaicins

HPLC: High-Performance Liquid Chromatography

PSII: Photosystem II

ME: mono-ester

DE: di-ester

EU: European Union

IAEA: International Atomic Energy Agency

kGy: kilo Gray unit

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

ESI: Electrospray Ionization

PTFE: Polytetrafluoroethylene

1.0 INTRODUCTION

1.1 Background study

Pepper (*Capsicum species*) is a genus of the Solanaceae family and is commonly divided into two significant groups known as pungent and non-pungent, one of the oldest, most popular, beneficial, and economical vegetable spices in the whole world. Chilli pepper fruits *(Capsicum sp.)*, probably ranked among the most consumed spice, are continuously increasing across the entire world, which has improved livelihood and income resources (Dessie et al., 2019). Hot pepper is commonly grown and consumed in Countries like China, Korea, and many other places for its nutritional components such as antioxidant compounds like carotenoids and vitamin C (Howard et al., 2000). The record indicates that chilli peppers' history can be traced to Mexico's several locations (Kraft et al., 2014) and other prominent pepper-growing countries. Paprika is used in many traditional foods in Hungary. It is one of the essential spices in such famous Hungarian dishes as the chicken paprikash or traditional soups. Hungarian paprika is perfect for giving meals a nice red colour; moreover, its taste is a significant part of traditional and modern cultural cuisine (Smith & Jusztin, 2014). Hot peppers contain capsaicinoids that give the fruit its pungency and its pharmacological attributes (Thiele et al., 2008). *Capsicum species* contain different sources of antioxidant compounds, including capsaicinoids (Ochi et al., 2003; Topuz & Ozdemir, 2007) and flavonoids, which are a type of phenolic compound (Materska & Perucka, 2005). Chilli is a good source of ascorbic acid, vitamin E (tocopherol), and provitamin A, which gives the red colour in pepper when the pigment is synthesised during ripening (Daood et al., 1996; Matsufuji et al., 1998). The composition of antioxidants in chilli peppers is dependent on genotype, maturation stage, harvesting period, postharvest handling, processing, weather, and storage conditions (Deepa et al., 2007). Peppers are medically beneficial due to their valuable biochemical compositions and considerable amounts of vitamins, phenolic compounds, and carotenoids (Howard et al., 2000). Chlorophylls and carotenoids are the most abundant pigments in nature and have a significant role in photosynthesis, the fundamental process of life on the earth (Green & Durnford, 1996; Gross, 2012; Kalt, 2005). Aside from peppers, other vegetables such as tomatoes contribute to a significant daily intake of antioxidants in diets due to their red colour pigment called lycopene (Salehi et al., 2019; Shi & Le Maguer, 2000).

Vitamin E concentration found in the pericarp of 32 pepper tissue accessions had α- and β-tocopherols which varied between their accessions, but α-tocopherol was always the predominant

form; and provitamin A levels that ranged from traces to 18.52 mg/100 g fresh weight (Baenas et al., 2019; Howard et al., 2000).

Several factors affect crop production and productivity, of which climate is one of the primary contributing determinants of yield and is expected to influence crop quality. Chilli pepper crop requires the right and precise amount of water for high yield and fruit quality. The use of drip irrigation is an opportunity for precise water application and nutrients or fertigation to crops. Abiotic factors can strongly influence an increase or decrease in crop yield. Changes in climatic factors such as temperature, precipitation, and extreme unexpected situations like drought, floods, and windstorms directly affect crop yield (Thornton et al., 2014). Photosynthetic activities are influenced by many factors such as the leaf's position, stage of development, light intensity, and composition (Kim et al., 2016; Li et al., 2020).

Spices such as red peppers are frequently exposed to insects and microorganisms during cultivation and storage, which may be potential contamination sources in foods even when added in small amounts (Schweiggert & Schieber, 2007; Taylor et al., 2010). One of the oldest techniques to prevent the deterioration of chilli is drying, especially sun drying. There are different physical pepper processing methods used by various food industries, usually dehydration, heating, canning, and cooling. The use of the hot drying preservation method affects macronutrients which degrade micronutrients, polyphenols, flavonoids, antioxidants, and spicy hotness while freezing preserves sensorial attributes, vitamin C, chlorophyll, and carotenoids composition (Barrett & Lloyd, 2012; Menichini et al., 2009). Dried peppers are less sensitive to irradiation than fresh ones. Their irradiation has been authorised at a maximum dose of 10 kGy and 30 kGy in Korea and the United States, respectively (Olson, 1998). Irradiation of dried spices is widely recognised and legally accepted in at least 51 countries worldwide, with a maximum overall average of 10 kGy (IAEA, 2008).

1.2 Problem statement

- Fresh pepper is highly susceptible to spoilage in less than three days.
- Aside from food, the growing demands of peppers by pharmaceutical industries require suitable breeding technologies of cultivars.
- The climatic problem in Hungary forces producers to shorten normal ripening by irradiating chilli peppers to reduce the growth of mycotoxins.

- Irradiation process that is done before fruit ripening is expected to be within acceptable dose rate, reduce microbial growth and at the same time keep phytonutrients quality.

1.3 Objectives to achieve

The main objective of this study is to establish how the effect of water supply on physiological factors of four (4) different chilli pepper (*Capsicum* sp.) cultivars grown under three different irrigation treatments and the response to gamma irradiation of phytonutrients in three (3) chilli pepper cultivars.

Specific objectives are:

1. To study the important physiological factors affecting different chilli pepper cultivars
2. To study the water supply and marketable yield of the various chilli pepper plants
3. To characterise by recent analytical protocols, the phytonutrients (vitamin C, capsaicinoids, carotenoids, and tocopherols) in the chilli peppers
4. To study the response of phytonutrients to gamma irradiation treatments and change over-ripening stages with retaining the levels of phytonutrients at acceptable measure.

2.0 LITERATURE OVERVIEW

2.1 Global economic importance of chilli peppers

The consumption of chilli peppers fruits *(Capsicum sp.)* is ranked among spices that have continually increased across the entire world, of which its production has doubled in size within a decade. According to FAO evaluation of chilli peppers', the world production statistics estimated a global chilli pepper production of 38,415,621 tons in 2016 in the cultivated territory of 3,737,635 ha, which increased to 4,255,050 tons (FAO, 2018). Red pepper is the world's second most important vegetable, ranked after tomatoes, and it is the most produced spice for food which provides essential vitamins and minerals (Guzmán & Bosland, 2017). The nutritional value of red pepper merits special attention due to its high vitamin A and E vitamin content. There are several varieties of peppers cultivated around the world. India is the largest producer of chilli peppers globally, with a contribution of about 25% of the world's production, followed by China. Production of dried chilli peppers in India is about 1605000 tons from 760000 ha area and green peppers 678000 million tons green peppers from 43000 ha area. The production of chilli pepper for spice, vegetables, and other uses increases every year (Olatunji & Afolayan, 2018; Sherman & Hash, 2001). It is estimated that it is annually cultivated on more than 1.5 million hectares in numerous countries. Forty-six percent of production is in Asia and, which makes Asia the highest producer of chilli peppers and its product (fig. 1). Southern Europe is the second most important producing region, with 24% of world production. The countries with harvest areas of more than 70,000 ha are China, India, Indonesia, Mexico, Korea, Nigeria, Ghana, and Turkey. However, the global production of peppers was recently estimated at 14.4 billion dollars (FAO, 2014). Due to the globally growing demand for pepper fruits, it is vital to consider several strategies to increase crop production and fruit quality or promote the investigation to improve plant resistance to environmental stresses (Bita & Gerats, 2013; Garcia-Mier et al., 2014).

In the past years, agriculture provided a 52% share of the positive balance of the Hungarian national economy. The rate of agricultural exports within the national economy's total exports was 8.3 percent in 2018, and the agricultural sector contributed EUR 2,869.3 million to the EUR 5,557.5 million surpluses of the national economy. Between 2010 and 2018, agricultural exports and imports increased by 48.6 and 56.7 percent, respectively, as a result of which the balance grew by 34.5 percent (Hungarian Central Statistical Office, 2019). Hungary is a player in the bell pepper market either on the import or export sides; Hungary shares in EU value exports of bell pepper

reached 1.2% in 2017 and ranked number 9th among top exporting countries in the EU. The Hungarians are known as intensive consumers of peppers; it is a significant component in their food and different meals (Bell Pepper Market Overview in Germany, 2018).

On the other hand, Hungary's share in EU imports of bell pepper was around 0.6% in 2017 and reached 14.5 million United States Dollars (USD). Hungary's imports of pepper have increased from 5,000 tons in 2012 to 9,275 tons in 2017. Over the last five years, the imports increased annually on average by 2.1%. The majority supply of pepper to the Hungarian market mainly comes from the EU markets; that boomed in 2014 and reached 78% of total Hungarian quantity imports of pepper. Hungary is the sixth biggest exporter of bell pepper among the EU countries with a share of 2% of total EU export quantity. Hungary imports of pepper have increased from 5,000 tons in 2012 to 9,275 tons in 2017. The import peaked in the year 2015 when the imported quantity reached 11,668 tons. Over the last five years, the imports increased annually on average by 2.1%. In 2017, *Capsicum*'s global production reached approximately 36 million tons of fresh fruit, and China had the highest production worldwide, 17,821,238 tons, followed by México with 3,296,875 tons (Hernández-Pérez et al., 2020).

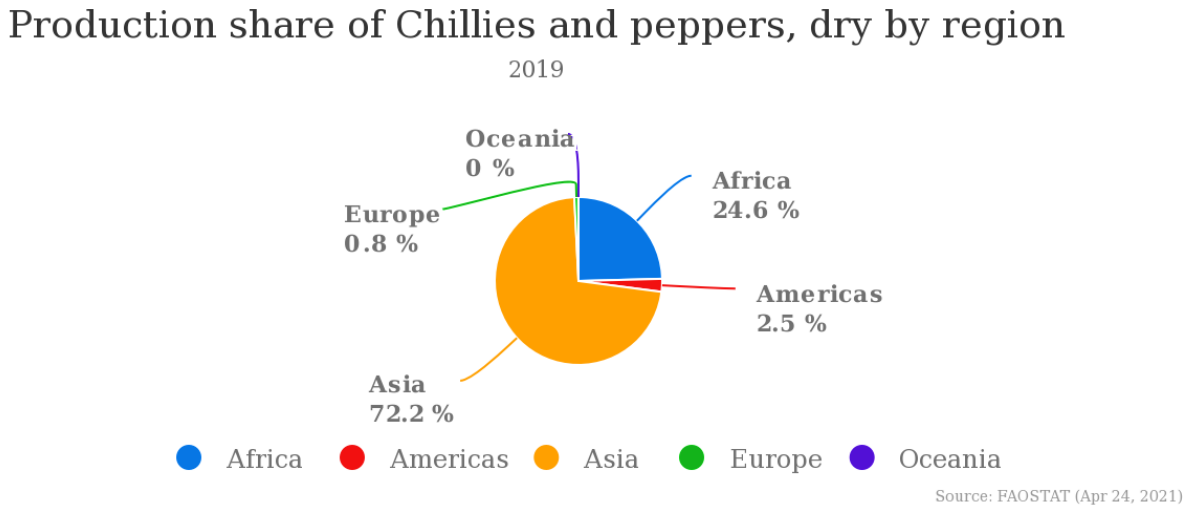


Figure 1. The world production ratio of peppers per continent according to FAOSTAT.

2.2 Pepper production in Africa

In Africa, agriculture is one of the sectors that are significantly affected by climate change and variability. Climate change increases temperature, drought, and extreme weather conditions, which is more evident in Africa's southern part as a result of altering precipitation patterns (Dore, 2005; Mason & Jourbert, 1997). Production of pepper in tropical Africa is estimated at 1 million tons,

with Ivory Coast (175,758 tons) and Ghana (126,291 tons) as the largest producers in the West. As of 2019, Ghana, Ethiopia, and Ivory Coast were ranked among the first ten (10) producers of peppers globally (fig. 2). Some challenges affect crop productivity in Africa, and they include environmental degradation, soil erosion, which affects soil fertility, and increased agricultural risks. These problems threaten the societal goals of improving food, income, and nutrition security, especially in small- and large-scale farming. The southern part of Africa has been the worst affected area for these emerging pepper farming challenges (Makate et al., 2016).

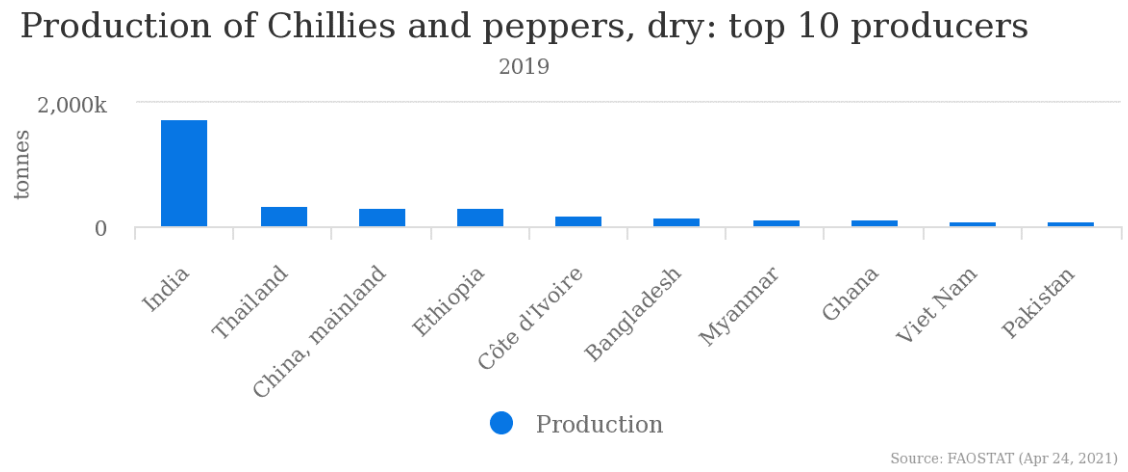


Figure 2. The top ten (10) highest producers of peppers in the world.

In Ghana, the three most essential vegetables grown are tomato, pepper, and onion. Pepper is considered an indispensable spice in Ghana, present in almost all dishes in Ghanaian homes. Pepper has been essentially cultivated for subsistence purposes in the past but has recently been seen as an export commodity due to its high demand, which is a critical tool for its economic growth. Ghana has six distinct agroecological zones based on climatic conditions and soil types. These are the Guinea savannah zone, Forest-savannah transition zone, Semi-deciduous forest zone, Sudan savannah zone, Coastal savannah zone, and the Rain forest zone (moist and wet evergreen) (Villano et al., 2019). Pepper in Ghana, for instance, is cultivated in all the ecological zones of the country; coastal savannah, rain forest up to the guinea savannah zones in open fields, and by extension, the use of greenhouse technology which is only applicable in research stations. However, few well-established farms have adopted greenhouse conditions for the production of various vegetables. Due to adverse environmental conditions, the Government of Ghana extended greenhouse technology on large-scale production when, in 2017, the planting for food and jobs policy was launched. So far, this policy, as an innovative mechanised programme, covers the

production of peppers, tomatoes, maise, and rice (Tanko et al., 2019). In Ghana, chilli peppers are classified within the scope of non-traditional exports. However, non-traditional exports have been described by the Government of Ghana as a pivotal contributor to Ghana's strategy to achieve middle-income status by the year 2020. Although the traditional exports of cocoa and gold are still vital for economic growth and foreign exchange generation, export diversification is required for accelerated economic growth. Some varieties of peppers cultivated in Ghana include Legon 18, Bird's eye, M12, California wonder, scotch bonnet.

2.3 Morphological composition of chilli peppers

Peppers undergo morphological, physiological, and metabolic changes during ripening, affected by genotype or variety, age of the plant, and growth conditions (Gómez-García & Ochoa-Alejo, 2013). Chilli pepper *(Capsicum sp.*) with about 25 species originated from Central and South America and Mexico. The essential and well-known species of chilli peppers are *Capsicum annuum,* which includes cayenne, jalapeno, bell, yellow wax. *Capsicum frutescens* with tobacco capsicum, a well-known variety, *Capsicum chinense* Jacq., include habaneros and scotch bonnet; *Capsicum pubescens* mainly pod-like types of pepper and *Capsicum baccatum,* mostly Yellow Peruvian Pepper (Araceli et al., 2009). Wild species of chilli peppers have seeds and erect fruits with an intense red colour and are highly pungent. Ripe pepper fruits belonging to different varieties display a range of colours from yellow, brick-red, to deep red (Stummel & Bosland, 2007). Several pepper species have been domesticated to produce different cultivated types at this stage, which are usually comprised of good taste and flavour, ranging from mild and sweet to hot and highly pungent. Hot peppers vary widely in their level of pungency. Some of the hottest cultivars are found in C. *chinense*, including 'Habanero', 'Red Savina,' and 'Bhut Jolokia' with pungency levels up to more than one million SHUs (Bosland & Baral, 2007). *Capsicum annuum* has the most significant variation in form, size, fruit colour, and shape, mostly elongated, round, and triangular. Pepper hybrid breeding is done by developing F1 hybrids, which is the easiest and simplest way of matching different types of dominantly inherited resistance and consideration of other traits like improvement of yield (Csilléry, 2006). It is essential to consider breeding techniques which are pests and insect resistance, for higher chilli yield (Pickersgill, 1997; Ravishankar et al., 2003).

Nowadays, sweet and pungent peppers are primarily used either as a vegetable for garnishing or as a hot spice in a meal. Aside from their uses as a vegetable or spice, peppers are vital for traditional or herbal medicine production. The phytochemical concentration in pepper

fruits has considerable amounts of vitamins, phenolic compounds, and carotenoid components that are influenced by genotype, stage of maturity, environmental and postharvest conditions (Alvarez-Parrilla et al., 2011; Siddiqui et al., 2013). Pepper fruit contains high levels of vitamin C, tocopherols (vitamin E), β-carotene content, and other carotenoids (Daood et al., 1996; Hornero-Mendez et al., 2000).

2.4 Pepper cultivation

Growing season and weather are important factors for pepper cultivation since this spice is a light-demanding species. It requires summer and autumn rich in sunlight to produce a good quality crop and grows at an altitude ranging from 1400 to 2100 m with about 600 - 650 mm rainfall. Pepper is considered a self-pollinating crop, although cross-pollination may occur. It is a herbaceous perennial crop that survives and produces yield for several years in tropical climates but is cultivated as annuals due to its sensitivity to frost (Kelley et al., 2009). Peppers adapt well to hot climates with an optimum seed germination temperature of 25-30°C. The temperature under 15°C or higher than 32°C may cause growth retardation, blossom-end rot, deficiency in fruit-set emergence, and a possibility of low yields (Kelley et al., 2009). The soil particles' arrangement determines soil structure in terms of texture, water-holding capacity, water, and air permeability. Pepper plants can be cultivated by direct seeding or transplanting after initial propagation by raising seedlings in nursery trays or boxes when plants are started in greenhouses or hotbeds in many production areas or outdoors seedbeds in mild-climate areas (Pittenger, 1992).

Peppers can be cultivated under open field conditions, in greenhouses, and in polytunnels. Chilli pepper plants are transplanted when they are 6-8 weeks old. Before open field planting, plants should be hardened but not excessively. Breeding of pepper or genome traits modification could facilitate higher production and better fruit quality. Genetic variability within a species is a valuable tool for screening and breeding for drought tolerance. Plant growth is generally enhanced by applying three primary macronutrients, namely nitrogen, phosphorus, and potassium. The application of nitrogen fertilisers, known to increase plant foliage, may reduce the light intensity in shaded plant parts. The excess use of nitrogen fertilisers may increase NO^3 concentration, which affects plant growth (Mozafar, 1993). Lack of phosphorus in the soil induces root proliferation and early crop maturity, affecting crop yield (Acquaah, 2009). Peppers are affected by diseases and pests when cultivated in open fields and greenhouses. Pests include insects, mites, nematodes, rodents, slugs and snails, birds. It is estimated that about 26 to 40% of the world's crop production is lost due to pests and diseases even though farmers practice crop protection activities (Oerke,

2006). Chilli peppers are infected by fungal, bacterial, and viral diseases, contributing to severe economic losses. Anthracnose is one of the most important diseases affecting chilli pepper production globally (Saxena et al., 2016). One common physiological disorder of pepper is blossom-end rot, a calcium deficiency disorder that appears only at the fruit's blossom end. The use of adapted varieties combined with careful crop management practices, notably the control of damaging root factors and proper irrigation and nitrogen fertilisation, helps control the effects of abiotic constraints (Hochmuth & Hochmuth, 2009).

2.4.1 Abiotic stress in pepper cultivation

Several factors affect crop production and productivity, of which climate is the primary contributing determinant of yield and is expected to influence crop quality. Factors that affect chilli cultivation could be biotic or abiotic. Abiotic stress factors include extreme temperatures such as heat, cold, freezing, alternating irradiation resolution, flooding or waterlogging on the field, drought, inadequate or low mineral nutrients in the soil, and excessive soil salinity. Abiotic stresses about the climate and soil (moisture and nutrients content) may add to biotic constraints and lead plants to stress and undergo anatomical and physiological disorders. Stressing the soil and its poor management may lead to rapid degradation, which eventually affects crop yield (Gruhn et al., 2000). Inadequate supply of soil nutrients, major soil nutrients like nitrogen, phosphorus, and potassium, when are not evenly distributed in the right proportion, may affect plant survival under environmentally stressed conditions (Cakmak, 2005). Soil nutrients distribution can be done through fertigation, which is very useful and economical in pepper cultivation (Abayomi et al., 2012; Kumar & Dey, 2011).

The use of drip irrigation is an opportunity for precise water application and nutrients or fertigation to crops (Mali et al., 2017). Chilli pepper crop requires a good and precise amount of water for high yield and fruit quality. The drip system has proven to be better than other conventional irrigation methods, especially in vegetable crop production (Maisiri et al., 2005). The use of efficient water through drip irrigation of bell peppers cultivated under open field conditions in the Mediterranean area produced quality fruits and yield (Sezen et al., 2006). Water deficit or water stress during flowering and fruiting can affect paprika fruit development (González-Dugo et al., 2007; Jaimez et al., 1999). Generally, pepper plants are sensitive to water deficit due to big leaf areas and higher stomatal conductance (Delfine et al., 2001). Low fruit yield is usually a result of reduced leaf area, plant growth, stomatal conductance, assimilation rate, water use efficiency, fruit yield and quality, leaf relative water content, and lack of macro-nutrition (Cantore et al., 1999;

Kirnak & Naim Demirtas, 2006). Stomatal closure and decreased transpiration rates are prompt responses to drought stress because they reduce plant tissues' water capacity and cause flower abscission (Aloni et al., 1991). Temperatures above 24°C may cause reductions in photosynthesis and leaf conductance (Da Matta et al., 1997). In the pepper production industry, drought imposes huge reductions in crop yields and quality, with significant economic losses of up to 70%. Water stress tolerance in pepper (*Capsicum annuum L.*) cultivars were exhibited by the better photosynthetic rate and reduced oxidative stress, mainly due to higher photorespiration, non-photochemical quenching, cytochrome, and alternate oxidase respiration (Hu et al., 2010).

The threat of abiotic stress is getting increasingly alarming as a result of population growth and climate change with expected greater adverse effects in vulnerable regions such as semi-arid West and Central Africa (El-Beltagy & Madkour, 2012; Thomas, 2008). Temperature and drought stress may cause oxidative stress, resulting in the differential synthesis of pepper fruit carotenoids across different environments. Given the inherent complexity of drought or heat stress tolerance, it is argued that a trait-based breeding approach would be helpful in the development of abiotic stress-tolerant varieties, which will be simplified by targeting important related traits that contribute to abiotic stress tolerance and understanding the genetic mechanism will be necessary for improving drought or heat tolerance. Drought-stressed pepper plants expressed a decrease in leaf water potential, implying a difference in plant water status (González-Dugo et al., 2007). In as much as some plants do thrive under water stress conditions, there are certain negative effects like reduction in growth rate, which will eventually impact parameters like the number of fruits and fruit size per plant. About 25% of water deficit drip irrigated for cultivated red peppers in the Mediterranean region produced high yield and quality fruits (Sezen et al., 2014). Leaf chlorophyll concentration is an important parameter that is usually measured as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content as well as plant health in general. Chlorophyll concentration or green pigment in a leaf is affected by several factors such as nitrogen in plants (Dong et al., 2019; Netto et al., 2005). These transmittance values, typically between 0.0 and 50.0, are proportional to the amount of chlorophyll in the sample which is a relative SPAD meter device that is used to derive chlorophyll values (Limantara et al., 2015; Uddling et al., 2007). Abiotic stress can significantly affect the photosynthetic activities of pepper plants, and therefore the use of chlorophyll fluorescence measurements can give a clear idea of plant health (Gorbe & Calatayud, 2012; Sharma et al., 2020). Measurement of chlorophyll and fluorescence is a simple, accurate, and non-destructive technique widely used in the investigation of damage or repair caused by various types of stress in the photosynthesis plant system (Rolfe & Scholes, 2010; Urban

et al., 2017). Abiotic responses can subsequently have an impact on the commercial or yield value of the pepper crop.

2.4.2 Biotic factors in pepper cultivation

Plant diseases have been a cause of significant crop losses worldwide. Economic losses caused by diseases are primarily due to lower fruit quality and marketability. Several pathogens, including viruses, bacteria, and fungi, are a nuisance in chilli peppers' cultivation and growth. Anthracnose, *Phytophthora* blight, damping off, downy mildew, blossom-end rot, fruit, and root rot are the most dreadful diseases of *Capsicum species* that cause severe yield loss. Anthracnose can be found in many hosts, including vegetables, cereals, legumes, perennial crops, and tree fruits. The disease can occur on leaves, stems, and fruit of host plants, causing necrosis and significant loss in productivity (Sutton, 1992). Infected fruits are not harmful when consumed by humans or animals, even though badly affected fruits with blemishes are usually not recommended for consumption. Chilli products affected by anthracnose are mainly rejected for their unpleasant colour and taste since they do not meet consumer preference. The minimal application of fungicides can effectively control anthracnose. Too many fungicides may pose a hazard to the environment and beneficial soil microorganisms (Singh et al., 2011).

Biological or chemical measures, cultural control, and resistant cultivars are beneficial for controlling anthracnose diseases. *Phytophthora* blight disease usually affects all parts of a red pepper plant at any stage of its growth. Pepper disease symptoms include root and crown rot, aerial blight in leaves, stems, and fruits (Lee et al., 2008). *Phytophthora* blight disease is spreading rapidly. It is responsible for the significant loss to growers, which has been estimated to account for 30–80% of the total global annual red pepper production loss (Savary et al., 2012; Vurro et al., 2010). This disorder can be controlled by mechanical, chemical, and biological measures. The use of chemical methods is mostly used in farming communities. Damping-off and root rot affect young plants, fruits, and emerging pepper seeds. Controlling these pathogens can be done by cultivating crops in soils containing compost and biological methods (Kim et al., 1997; Whipps, 1997).

Both sweet and pungent peppers cultivated in open fields and greenhouses are affected by insects and pests. They include thrips, aphids, beetles, spider mites, whiteflies, caterpillars, nematodes. Thrips have piercing mouthparts used to burrow and feed on new pepper leaves and under closed areas in flowers to destroy them (Weintraub, 2007). Whiteflies and spider mites affect peppers by feeding directly on plants, which decreases the area of photosynthetic activity and

induces leaf abscission in extreme infestations (Rabbinge, 1985). These insects and pests can be controlled by using natural enemies or predators such as *Orius insidiosus* against thrips for field cultivated peppers (Funderburk et al., 2000). The use of insecticides as fumigants or foliar to control whiteflies and two-spotted spider mites in glasshouse or greenhouse pepper cultivation (Gorman et al., 2002). The use of predatory mite, *Neoseiulus cucumeris,* to control broad mites in sweet peppers (Weintraub et al., 2003).

2.5 Chemical composition of phytochemicals

2.5.1 Capsaicinoids and their significance

Capsaicinoids are responsible for the hot and robust taste of pepper fruits, known for their pungency and pharmacological attributes (Luo et al., 2011). Capsaicinoid concentration or the pungent taste of pepper fruits is attributed mainly to its flavour, which acts as a spice when used as food. Chilli pepper also contains oleoresins, which gives the chilli pepper its aromatic odour (Jin et al., 2009). They are a group of alkaloids synthesised in the interlocular septum and the placental tissue (Curry et al., 1999). Capsaicin and dihydrocapsaicin are the two significant capsaicinoids (more than 90%) found in the fruit pericarp (Bosland et al., 2015), placenta (Suzuki et al., 1980), and seed tissues of all peppers; nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin are present in lower amounts(Govindarajan et al., 1987; Kurian & Starks, 2002). Total capsaicinoid concentration comprises 60% capsaicin and 30% dihydrocapsaicin (Topuz & Ozdemir, 2007). The chemical structures and pungency levels of capsaicinoid have been summarised from previous findings (fig. 3) according to Ravishankar et al. (2003) and Eich (2008). Capsaicin is formed by a volatile, pungent, hydrophobic, colourless, and odourless structure with a polar amide group ($C_{18}H_{27}O_3N$).

Levels of capsaicinoids can vary from one hybrid to the other, ripening stage or stage of maturity at harvest and storage and processing of these pepper fruits (Iqbal et al., 2013). Due to the globally growing demand for pepper fruits, it is essential to consider several strategies to increase crop production and fruit quality or promote the investigation to improve plant resistance to environmental stresses (Garcia-Mier et al., 2014). High exposure of capsaicinoids to intense light may reduce its pungency level and increase when introduced to a shaded environment, as reported (Gurung et al., 2011; Nakarin Jeeatid et al., 2017). There are several major enzymes involved in the metabolism of capsaicin; one of them is peroxidase which occurs in the placenta and outer layer of pericarp epidermis cells. Peroxidase activity decreases when capsaicinoid concentration increases. The amount of capsaicin in hot peppers also varies significantly between varieties and

is measured in SHU. The world's current hottest known pepper as rated in SHU is the Trinidad Moruga Scorpion, which has been measured at over 2,000,000 (Hernández-Pérez & Gómez-García, 2020).

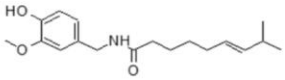
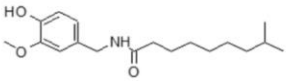
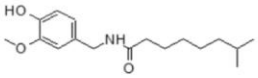
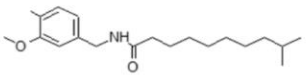
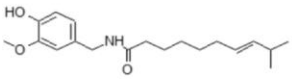
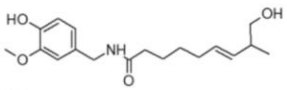
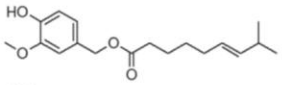
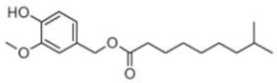
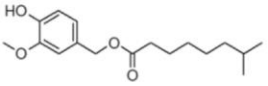
compound	typical relative amount	scoville heat units (SHU)	chemical structure
capsaicin	69%	15,000,000	
dihydrocapsaicin	22%	15,000,000	
nordihydrocapsaicin	7%	9,100,000	
homodihydrocapsaicin	1%	8,600,000	
homocapsaicin	1%	8,600,000	
ω-hydroxycapsaicin		---	
capsiate		---	
dihydrocapsiate		---	
nordihydrocapsiate		---	

Figure 3. The chemical structures and pungency levels in peppers (Eich, 2008; Ravishankar et al., 2003).

Levels of total capsaicinoids can be converted to Scoville heat units, a measurement for pungency developed by Wilbur Scoville (Zachariah & Gobinath, 2008). The number of Scoville units is equal to the average number of times the pepper extract will have to be diluted in order for the pungency to be imperceptible. By description, one part per million (ppm) of capsaicin has a pungency of 15 SHUs (Peña-Alvarez et al., 2009). Scoville categorised four pungency groups: mild (0−5000 SHU), medium (5000−20 000 SHU), hot (20 000−70 000 SHU), and extremely hot

(70 000−300 000), as shown in fig. 4. Another pungent cultivar is “Naga Viper”, which has more than 1.3 million SHU (Wahyuni et al., 2013).

Scoville heat unit (SHU)	Category	Type
>80,000 SHU	Very highly pungent	Habanero 100,000 to 300,000
25,000-70,000	Highly pungent	Chile piquin 30,000 to 50,000
3,000-25,000	Moderately pungent	Serrano 5,000 to 15,000
700-3,000	Mildly pungent	Anaheim 500 to 1,000
0-700 SHU	Nonpungent	Pimiento 0

Figure 4. Pungency of *capsicum* varieties in Scoville heat units (Hernández-Pérez & Gómez-García, 2020).

Capsaicinoid compounds are known for their therapeutic results on gastric ulcers and rheumatoid arthritis (Satyanarayana, 2006). Among spicy food ingredients, chilli peppers, as epidemiological studies have demonstrated to have nutraceutical potential as an anti-inflammatory, analgesic, blood glucose regulation, and antioxidant agents. Capsaicinoids showed anti-atherosclerotic, antidiabetic, anti-obesity, and antihypertensive activities (Baenas et al., 2019; Kwon et al., 2003; Rupasinghe et al., 2016). Capsaicinoids also have pharmacological properties, with a high content of capsaicin being medically beneficial in treating painful conditions such as cluster headache, painful diabetic neuropathy (Tsuchiya, 2001). Capsaicin, known to be an active compound in chilli peppers, is currently used to treat osteoarthritis, post-herpetic neuralgia, and psoriasis (Zhang et al., 1994). It is also noted for decreasing myocardial, aortic cholesterol levels and obesity even when consumed in low amounts (Kempaiah et al., 2005). Currently, capsaicinoids have been studied and found to be an effective treatment for several human nervous disorders, including cystitis, rheumatoid arthritis (Mason et al., 2004), and human immunodeficiency virus (Perucka & Materska, 2001; Robbins, 2000). Hot chilli peppers cause high salivation, participate in indigestion, and have a laxative effect. Capsaicin has antioxidant, antimutagenic, anticarcinogenic, and immunosuppressive properties (Jin et al., 2009; Prabhat et al., 2010).

2.5.2 Vitamin C in chilli peppers

Vitamin C, also known as ascorbic acid ($C_6H_8O_6$), is a water-soluble nutritional constituent of pepper fruit, which is biologically known as an active compound that has antioxidant properties (Rietjens et al., 2002). High levels of vitamin C can be found in fully ripened pepper fruits. However, vitamin C levels in pepper fruit vary from pepper cultivars and species to others (Howard et al., 2000). Vitamin C is one of the most critical indicators for nutritive quality in many crops. It is generally known that pepper fruit is characterised by its highest content from all vegetables. The content of vitamin C in vegetables and fruits is mostly influenced by variety, preharvest climatic conditions, cultural practices, maturity, harvesting methods, and postharvest handling procedures (Pérez-López et al., 2007).

Vitamin C, most vital in fruits and vegetables, is necessary for human nutrition and has an essential function for the immune system, enzyme activation, reduction of oxidative stress, and many essential metabolic processes (Navarro et al., 2006). Vitamin C supports collagen formation and absorption of inorganic iron, reduces plasma cholesterol level, and strengthens the immune system (Bae et al., 2014). Almost 90% of vitamin C in human food is obtained from fruits and vegetables (Halliwell, 2001). Both hot and sweet peppers contain more vitamin C to prevent flu colds than any other vegetable crop (Li, 2008). Vitamin C is necessary for healthy skin and gum maintenance, as well as for the prevention of scurvy. It reduces the risk of cardiovascular diseases and some forms of cancers due to its high antioxidant activity (Harris, 2013)

2.5.3 Carotenoids in peppers

Carotenoid is a subgroup of isoprenoid compounds and currently contain more than 700 characterised structures. Since 1980, about 7500 papers on carotenoids have been published in various fields of study in chemistry, physics, food, biology, and medicine, ranging from natural colouring to profound physiological effects (Arimboor & Natarajan, 2015). The fruit colour is highly variable; unripe fruit can be green, yellow, or white, turning to red, dark red, brown, and sometimes almost black in the ripening stage (Matus et al., 1991). The different colours are mainly due to the carotenoid concentration; capsanthin, capsorubin, and cryptocapsin that produces red colour and aroma (Deng et al., 2018), while beta-carotene, zeaxanthin, violaxanthin, and beta-cryptoxanthin produce yellow to orange colour (Eggersdorfer & Wyss, 2018; Giuffrida et al., 2013). The intense red colour and pungency are considered to be the critical consistency parameters for the paprika trade.

Oxygenation is the fundamental cause of the degradation of carotenoids. The high degree of carotenoids' unsaturation makes them particularly sensitive to light, heat, and oxygen (Carnevale et al., 1980). About more than 30 different pigments have been identified in pepper fruits ranging from yellow and orange to red colours during ripening stages (Collera-Zúñiga et al., 2005; Deli et al., 2001). The pigment character of carotenoids, for which they are best known, is imparted to the colourless basal phytene structure by introducing additional double bonds in conjugation. Lutein was the most abundant at the immature stage, then decreased in further ripening stages and was absent in the red or deep red stages. The total carotenoid levels in red pepper varieties increase as they ripen (Ha et al., 2007). During pepper fruit ripening, P-cryptoxanthin, anthraxanthin, and violaxanthin contribute to the rapid synthesis of ketoxanthophylls (Davies et al., 1970; Deli et al., 1992). Capsanthin levels in red paprika were low in the early stages and increased gradually until the maturation stage (Deli et al., 2001). Carotenoid pigments or colour of red-pepper powder during processing is a serious problem and usually expected to deteriorate (Kim et al., 2004; Minguez-Mosquera et al., 1994).

Carotenoids are commercially incorporated as food colourants and feed additives mostly used in pharmaceutical, nutraceutical, and cosmeceutical industries (Berman et al., 2015; Mu et al., 2019). Carotenoids have antioxidants properties (Bartley & Scolnik, 1995), anticarcinogenic components and, are cancer chemo-preventive (Zhang et al., 1991). They are found in food derived from leafy vegetables, vegetable oils, and yellow-orange fruits that are mostly made up of lycopene, which can also be found in tomatoes (Rao & Rao, 2007).

Beta-carotene, one crucial component of carotenoids, can be found in different varieties of orange, yellow, and green fruits and vegetables like carrot, red chilli paprika, palm oil, sea buckthorn berries. They can also be found in fruits and vegetables such as tomatoes, watermelons, guava, and pink grapefruit, which contain lycopene (Khoo et al., 2011; Ranjith et al., 2006).

2.5.4 Tocopherols (Vitamin E)

Vitamin E compounds (tocopherols and tocotrienols) are well recognised for their effective inhibition of lipid oxidation in foods and biological systems (Burton & Traber, 1990; Sies et al., 1992). Lipid soluble α-tocopherol is the main vitamin E component known to be a thylakoid stabiliser and is mostly synthesised for reactive oxygen species (Shintani & DellaPenna, 1998). The antioxidant activity of tocopherols and tocotrienols is mainly due to their ability to donate their phenolic hydrogens to lipid free-radicals (Shahidi & Ambigaipalan, 2015). The principle for

measuring antioxidant activity is based on hydro peroxidation of polyunsaturated fatty acids through radical chain reaction (Terao et al., 1992).

Lipid oxidation is when degradation occurs in free radicals responsible for odours and flavours in fats, oils, and foods containing lipids, which affects the desirable qualities of food (German, 1999). Lipid oxidation is a significant cause for food quality deterioration, which decreases the nutritive value of food, their shelf life and alters their taste (Alamed et al., 2009; Shahidi et al., 1992). The α-tocopherol concentration in the fruit pericarp of the yellow and red pepper varieties of different origin was compared to red spice paprika with changes in the carotenoid and antioxidant concentration affecting quality (Koncsek et al., 2019; Márkus et al., 1999b). More recent research suggests that plastid α-tocopherol synthesis during leaf development, fruit ripening, and senescence is regulated at the level of mRNA accumulation of genes coded for the enzymes involved in forming tocopherol precursors (Holländer-Czytko et al., 2005). Tocopherols have significant antibiotic properties and can reduce cholesterol levels when consumed in the diet, even in small quantities (Conforti et al., 2007).

2.5.5 General composition of polyphenols, total phenolic and flavonoid concentration in peppers

Peppers contain various nutritional compounds such as flavonoids, polyphenols, and mineral elements (Chuah et al., 2008; Materska & Perucka, 2005). Mineral elements or bioactive compounds like phenolic compounds are an essential group of secondary metabolic structures synthesised by plants due to adaptation to both biotic and abiotic stress conditions such as infection, wounding, water stress, cold stress, and high visible light. According to Materska et al. (2003), two new flavonoids were identified in hot pepper fruit pericarp to examine their biological activity since the demand for hot peppers in recent times has increased compared to the previous years. Research on phenolic compounds are limited, but protective phenylpropanoid metabolism in plants has been well documented (Pichersky & Gang, 2000). Also, flavonoid content in pepper fruit concentration is mainly quercetin and luteolin that was developed as a result of the hydrolysis process (Hertog et al., 1992; Howard et al., 2000; Lee et al., 1995). Water stress induces the activation of the phenol biosynthetic pathway under deficit irrigation conditions, while mild water stress increased the phenolic content in pepper plants (Estiarte et al., 1994). Phenolic compounds have attracted research interest because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with the standard, natural metabolism of aerobic cells (Halliwell, 1996). Vitamin E has been proven to reduce sepsis, intracranial haemorrhage, and neonates (Fish et al., 1990).

2.6 Processing and preservation methods of peppers

There are many general methods of preserving food, such as canning, freezing, but drying is an important method used to preserve pepper. In this process, heat is used under controlled conditions to extract the moisture that is usually present in peppers. This operation aims to reduce the enzymatic action and the microorganisms that damage the shelf-life of products. Different types of chilli fruits may be produced throughout the year. In Hungary, spice pepper pods should naturally be over-ripened for 3-4 weeks to reach the technological ripeness stage, at which a maximum level of quality components is approached (Gnayfeed et al., 2001). Since their raw state is highly perishable, significant losses may occur from the production areas to the consumption centres. Therefore, it is necessary to process them into another form. Also, storage problems and negative transportation effects have made it necessary to improve their preservation (Jia et al., 2017; Sanatombi & Rajkumari, 2019). Another advantage of drying is that the criteria for packaging, storage, and transport costs are minimised.

Traditionally, chilli peppers are dried using either solar energy or direct sun exposure or mechanical heat drying; these methods require long-term exposure to temperature or high temperatures and short-term exposure. However, these factors render the fruit to undergo structural, chemical, and nutritional changes, affecting their nutritional composition, bioactive compounds, and quality attributes such as taste and colour (Kowalski et al., 2013). Usually, pretreatment techniques, such as blanching, the immersion of fruits in hot water at various times, and chemical procedures are encouraged before drying (Deng et al., 2019). Subsequently, when dried pepper becomes vulnerable to fungal attacks and mycotoxins, its taste and aroma become undesirable. Quality deterioration of chilli peppers also occurs during storage due to oxidative processes that result in changes in the colour pigments (Öztekin et al., 2006).

Application of decontamination methods, such as ethylene oxide fumigation, steam heat sterilisation, and irradiation, has been used to extract such contaminants. Irradiation is mostly accepted for these decontamination treatment methods, as steam heat sterilisation is limited and prone to re-contamination before packaging or storage. In addition to the decontamination process limitations, ethylene oxide fumigation is restricted in several countries due to the possibility of toxic residues (Diehl, 2002; Farkas, 1998). However, irrespective of the processing methods, red pepper is sensitive to fungal contamination and aflatoxin formation (Coksoyler, 1999). Chilli peppers introduced to long-term storage can expose them to insect pest attacks, which may reduce the nutritive value in the product, affect their handling properties, and contamination, rendering

them unfit for trading or consumption. Climatic conditions, usually in the tropics, are more favourable for mycotoxin production (Montoya-Ballesteros et al., 2014).

2.6.1 Drying of peppers

Drying is the primary method used in food preservation and is usually done by introducing food products to heat under controlled conditions to remove the water normally present in them (Sagar & Suresh Kumar, 2010). Drying lowers water activity, which prevents enzymes but does not contribute to total inhibition. Drying is done to reduce the activity and multiplication of enzymes and microorganisms that induce food spoilage and prolong their shelf-life (Díaz-Maroto et al., 2003). Other benefits of drying are reducing packaging and storage needs and transport costs (Biji et al., 2015). Drying can promote vitamin degradation, antioxidant activity reduction, and undesirable changes of colour, texture, and flavour of the fresh product (Crapiste, 2000). Temperature, moisture, and humidity can influence mycotoxins' growth in food products, which eventually affect its postharvest quality (Neme & Mohammed, 2017). There various processing methods for drying food products. The use of the sun in drying food products such as peppers is the oldest method of processing (Condorı et al., 2001). Besides, the use of solar-driers (Hossain & Bala, 2007), oven-drying (Noh et al., 2015), ultrasound drying (Chemat et al., 2011) are alternative methods of drying. In Hungary, spice red peppers and chillies are traditionally sun-dried. The farmers dry their peppers on the walls around their houses inside a mesh bag. For mass production, thermal drying with air current in drying tunnels is mainly used. Each method has advantages and disadvantages concerning quality, shelf-life, and food safety parameters. Drying may result in quality and colour degradation in paprika (Topuz et al., 2009). It has been found that quality attributes and antioxidant activity can be affected by the temperature used for drying (Ornelas-Paz et al., 2013; Vega-Gálvez et al., 2009). In previous work, non-pungent spice, red pepper varieties, and hybrids have been found to differ substantially in their response to drying conditions (Daood et al., 2014).

2.7 Food irradiation

Food irradiation is a procedure in which food is subjected to a precisely regulated amount of energy in the form of high-speed radiation. Food irradiation promises better and cleaner food to the public by reducing bacterial damage. Many agricultural goods are not marketed due to pest infestation and microbial degradation; the use of food irradiation has become a solution to all of these problems (Wilkinson & Gould, 1996). Interest in the irradiation process is rising due to persistently high food losses due to infestation, pollution, spoilage, growing worries regarding foodborne

diseases, and growing foreign trade in food items that must meet stringent import safety, quarantine requirements (Sádecká, 2017). The use of irradiation in the food production industry has been faced with major opposition by customer acceptability due to the procedure, despite the technology's substantial possible benefits (Henson, 1995).

Many studies have shown that irradiation is a safe process, and therefore in 1994, WHO declared that irradiation of food is safe from the nutritional and toxicological point of view. Challenges at the postharvest stage of spice peppers are toxification through increasing microbial growth and quality decrease during over-ripening and drying. To make spice pepper products acceptable, human consumption of over-ripened peppers must be controlled to avoid toxin contamination from the food safety point of view. When irradiation doses are optimised against *Aspergillus* in hot peppers, they effectively decontaminate them, preserves the quality of the pepper, and make them safe to consume (Weiss & Landauer, 2003). The application of irradiation dose of 1 kGy in high oxygen atmospheres may be evident as the most effective way to assure the elimination of both surface and internal contamination of crops by pathogens. The most significant current concern pathogens are *Salmonella* found in tomatoes, seed sprouts, and spices; and Escherichia coli O157: H7 on leafy vegetables like spinach and lettuce (Olaimat & Holley, 2012). Toxicity decrease can be achieved by either shortening the over-ripening or applying efficient physical or chemical treatments. Irradiation alone or combined with other treatments has been effective for detoxification of some foods, including spices. Toxicological and nutritional tests have confirmed that foods irradiated at doses below 10 kGy are safe to consume (Farkas, 1998; Smith & Pillai, 2004). Spices often derive in developing countries, where harvesting and storage conditions are inadequately managed for food quality. The United States of America Food and Drug Administration (FDA) has set a limit for irradiation treatment of culinary herbs, seeds, spices, vegetable seasonings, and blends of these aromatic vegetable substances that must not exceed 30 kGy (Bendini et al., 1998; Olson, 1998).

Food irradiation is an emerging modern technology in a number of countries where irradiated food intake is of major concern in the near future (Maherani et al., 2016; Thakur & Singh, 1994). Gamma irradiation is more effective than using ethylene oxide as a fumigant in controlling microbial contamination without any opposing consequence (Byun et al., 2002). The use of irradiation destroys bacterial contaminants, viruses, or aflatoxins but can cause significant losses of vitamins, particularly E and B1, and fatty acids in high-fat foods (Henson, 1995). In dried fish products, a dose rate of 2.5 kGy effectively eliminates harmful microbes (Duah et al., 2018).

Irradiation of spice red peppers have proven to reduce microbial growth, particularly pathogens (Song et al., 2014) to minimise colour and quality loss during storage of powders (Topuz & Ozdemir, 2003), to control mould growth and mycotoxins (Byun et al., 2004), and to extend the shelf-life of spice peppers (Owoade & Ademola, 2012).

Food processors' principal concern is to ensure that microbial load in food ingredients and processing does not allow food spoilage and diminishes its microbial safety. Similarly, consumer attitudes to food irradiation and willingness to purchase irradiated food reflect the fundamental characteristics of the process itself and the social, economic, and political environment within which food products in general and irradiated food products are produced, purchased, and consumed. There is some evidence that consumers may not place a high value on the process's potential benefits, which might otherwise offset such concerns and support adoption. For example, increased shelf-life is a benefit of food irradiation, which has been emphasised by proponents of the process. However, there is little evidence to suggest that there is an identifiable demand for further increases in the shelf-life of food products (Gould, 1996). The spice decontamination process by gamma-radiation and other dry food constituents is a viable and effective alternative to other decontamination processes, which have a great deal of application potential in developing and developed countries.

The Gamma irradiation process is done by loading samples in a Cobalt-60 source (fig. 5). Samples are packaged into sterilised zip-lock bags or sacks and labelled according to the dose treatment. They are carefully loaded on the tray, and their movement on the rail is controlled from a technical control room as they move into the source direction. The samples, according to their dose treatments, are irradiated per source exposure time. The gamma irradiation source is stored in a pool of water to absorb energy emitted by the source and protect workers from radiation exposure. Prior to gamma-irradiation treatment, preliminary studies are conducted to determine the exact time, dose-dependent using dosimeters, and source strength (IAEA, 2002).

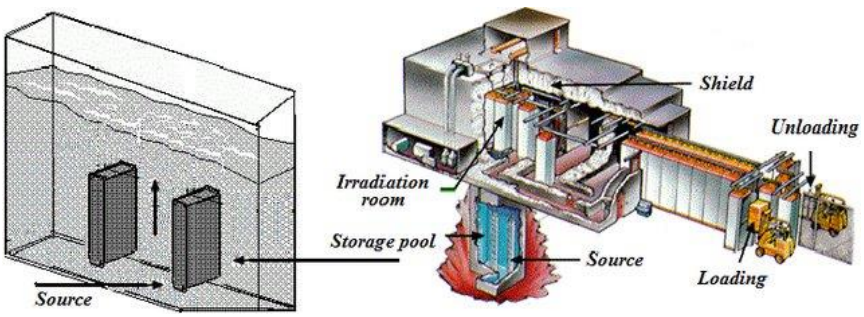


Figure 5. Gamma irradiation facility fitted with a Cobalt 60 source

3.0 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 characterised 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. Chilli 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 17, 2018, and May 13, 2019, each season against three (3) different water supply treatments; 0% (control except for natural precipitation), 50% deficit irrigation and 100% optimum water supply. 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 2018 and 1.5 m in 2019. 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 (fig. 6-7).

3.1.1 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 water supply, 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) (Allen et al., 1998; Takács et al., 2018) (Table 1, Fig 8). The trends of maximum and minimum accumulated precipitation and irrigation (varied between 340 mm – 620 mm in 2018 and 125 mm – 410 mm in 2019) at an average air temperature of 23.8℃ in 2018 and 24.8℃ in 2019 (fig. 8). 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 (3) different water supply treatments; control (0%) except for natural precipitation with no regular irrigation, deficit irrigation (50%), and optimum water supply (100%).

In 2018 between August and early October, the rainfall pattern changed with unexpected heavy rains recorded. The high amount of precipitation in 2018 showed lower mean temperature and relative humidity when compared to 2019 (Table 1). 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 fertilisation 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 (2018 and 2019), 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.

Table 1. Meteorological record and water supply throughout the chilli 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%
2018	23.8	71	347.8	132.6	272.2	347.8	480.4	620.0
2019	25.8	72.3	132.6	152.2	289.0	132.6	284.4	421.6

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

Figure 6-7. 2018 Experimental field after plant protection activities and 2019 Experimental field after the drip-irrigation system was set up, respectively. Credit: Stella Agyemang Duah

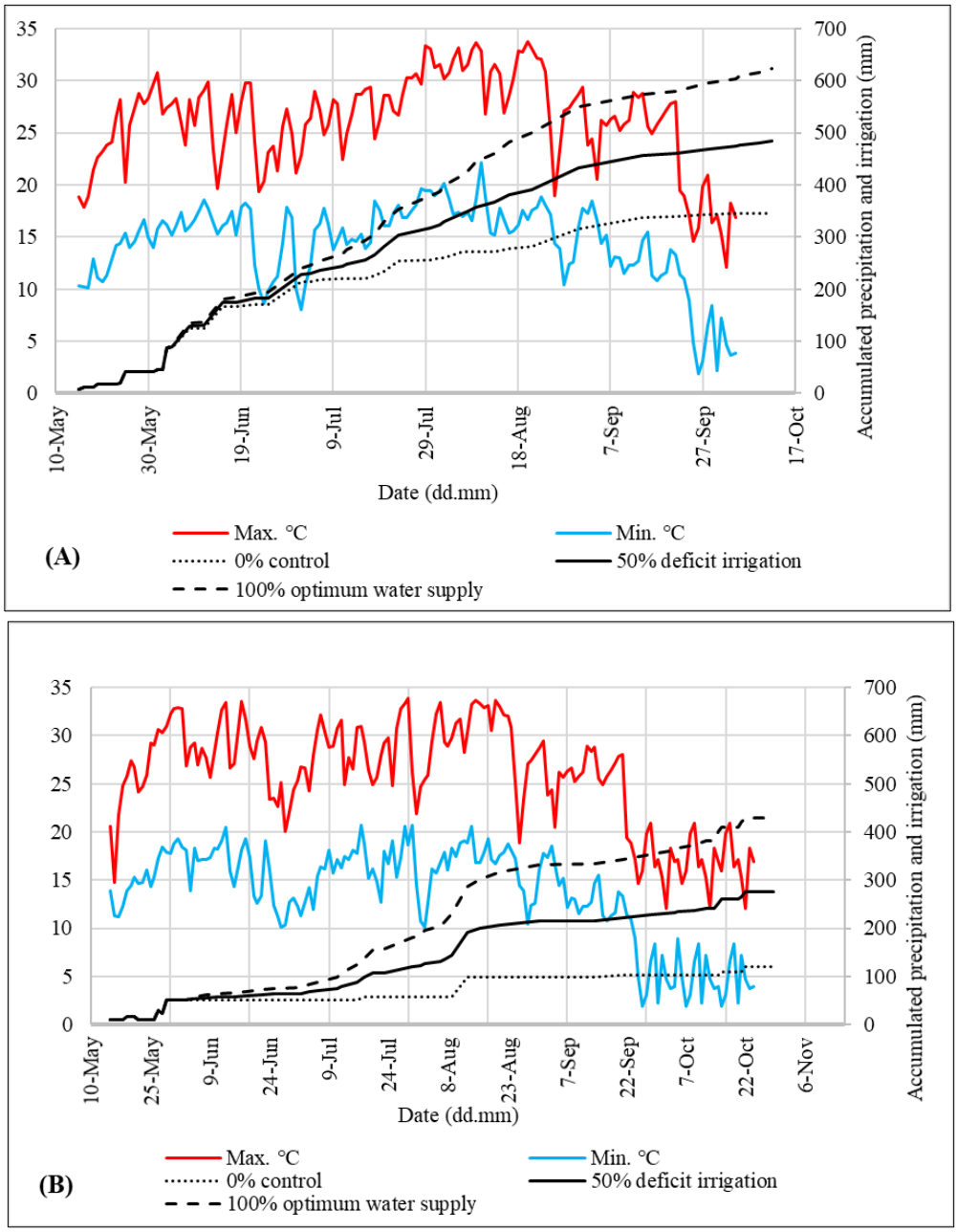


Figure 8. Trends of daily maximum (Max.) and minimum (Min.) air temperature (°C), and accumulated precipitation and irrigation (mm) for the growing seasons, dated May 05, 2018 – October 17, 2018 **(A)** and May 05, 2019 – November 11, 2019 **(B)**.

3.1.2 Plant materials

Chilli 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 capsaicinoid 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 (Tóth-Horgosi et al., 2019).

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 similar to the Unihot variety. The content of capsaicinoid 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 (*Capsicum annuum* × *Capsicum 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 capsaicinoid 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 *Capsicum 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 & Votava 2012).

3.2 Physiological responses

3.2.1 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.

3.2.2 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 is capable of measuring 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.

3.2.3 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 Goethem et al., 2013).

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

where Fo = initial fluorescence

Fm = maximal fluorescence

Fv = variable fluorescence (Fm–Fo).

3.2.4 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.

3.3 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**
2018	17 May	13 August
		03 September
		24 September
		15 October
2019	13 May	13 August
		10 September
		07 October
		28 October

3.4 Analytical measurements

3.4.1 Determination of total capsaicinoids

Total capsaicinoid concentration was determined and calculated as the sum of individual compounds (nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin derivatives, and homodihydrocapsaicin derivatives) that appeared on the chromatogram following the method of (Daood et al. (2015). About 3 grams of homogenised 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 subjected to 10 times (9:1) dilution process of 9 mL of chromatography grade methanol: 1mL filtrate for 'Hetényi Parázs' and 'Unikal' and purified through a 0.22 μm PTFE (Chromfilter) syringe into vails. 'Unijol' and 'Habanero' were subjected to 20 times (9:1,1:1) dilution process of 9 mL chromatography grade methanol: 1mL filtrate and filtered through a 25 μm Chromfilter (syringe) into a 10 mL glass beaker. The filtrate was further diluted using an Eppendorf pipette of 1 mL methanol and 1 mL filtrate (from syringe filter) into vails. All vails were injected into an HPLC column.

For this, extracts were diluted appropriately and injected into 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. The fluorometric capsaicinoid detection was carried out at two wavelengths (EX: 280 nm and EM: 320 nm). Peaks corresponding to the different capsaicinoid compounds were identified based on retention time and mass data from LC-MS/MS analysis as described in previous work (Daood et al., 2015) created by analysing the standard analytical material (Appendix 1).

3.4.2 Determination of vitamin C

Vitamin C content was determined according to the methods and HPLC protocols of Nagy et al. (2015). About 3 grams of homogenised pepper fruit (seed excluded) was crushed in a crucible mortar with quartz sand. 30 mL of 3% metaphosphoric acid solution was gradually added to the mixture and then transferred into a 100 mL Erlenmeyer flask with a stopper. 3% metaphosphoric acid was prepared by dissolving 30 grams of metaphosphoric acid crystals into 1 L of distilled water and ultrasonicated.

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).

3.4.3 Determination of carotenoids and tocopherols/ vitamin E

Carotenoids and Tocopherols were determined according to the methods and protocols of Daood et al. (2014). 2.5 grams of homogenised pepper fruit (seed excluded) from 'Hetényi Parázs', 'Unikal', 'Unijol', and 3.5 grams of 'Habanero' were respectively used in this experiment. Homogenised pepper fruit was crushed in a crucible mortar with quartz sand. 20 mL of methanol was added for 1-2 minutes and poured the upper into an Erlenmeyer flask. 10 mL of methanol (analytical grade) was then added to 50mL of dichloroethane in a 100 mL graduated cylinder and shaken gently. The mixture was poured into the remaining homogenised pepper in the crucible mortar and then transferred into the Erlenmeyer flask and shaken vigorously. Few drops of distilled water were added and shaken gently. The mixture was separated with a burette into a flat bottom flask using a filter paper containing sodium sulphate anhydrous in a separating funnel. 5 mL of dichloroethane is added to filtrate through the filter paper for further extraction and evaporated with a rotary evaporation chamber for 10mins at 70°C and 40°C vacuum, respectively. The flask was offloaded from the chamber tube after all filtrate evaporated. Using an Eppendorf pipette, 5 mL of pigment eluents and 5 mL of methanol (liquid chromatography grade) were respectively dropped into the flask and shaken evenly. This was done for Hetényi Parázs', 'Unikal', and 'Unijol'. For 'Habanero', 2.5 mL of pigment eluents and 2.5 mL of methanol (liquid chromatography grade) were used. An ultrasonic shaker was used where necessary to ensure that no residue is left in the flask. The filtrate was further passed through a 0.22 μm PTFE membrane syringe filter into vails and injected into the HPLC column.

Carotenoids peaks that were identified in this experiment were free capsanthin (Free caps), free zeaxanthin (Free zeax), capsanthin monoester (Caps ME), zeaxanthin monoester (Zeax ME), beta-carotene (B-carotene), capsanthin di-ester (Caps DE), and zeaxanthin di-ester (Zeax DE). Peaks classification was done based on pepper colour intensity (capsanthin and zeaxanthin) and their nutritional value (β-carotene). Total carotenoid concentration was calculated as the sum of all individual peaks identified on the chromatogram. 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.

Identification of all carotenoid compounds in the pepper cultivars was made using the liquid chromatography-diode array detection-mass spectrometry (LC-DAD-MS) as shown in Appendix 6. In the tandem mass spectrometry (MS/MS) detection and for the optimisation of the electrospray ionisation (ESI) source parameters, flow injection analysis (FIA) of all-trans- β-carotene standard was used. All experiments were conducted with positive ionisation mode with the following settings: the capillary voltage was 1.5 kV, nebuliser 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 characterised by the appearance of an extra absorption maximum between 340 and 362 nm and the value of Q-ratio (Lin & Chen, 2003; Schieber & 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.

On tocopherols, peak separation was performed 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 fluorescence (FL) detection at ex: 290 and Em:325nm. Tocopherol concentration peaks identified were 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).

3.5 Irradiation of dried peppers

In the first-year cultivation season, 'Hetényi Parázs', 'Unikal' and 'Unijol' peppers were collected at various ripening stages (brick-red and red), cut-through, oven-dried (70°C), milled into powder and were packaged into nylon sacks and vacuum sealed for γ-irradiation treatments with doses 0.5 kGy and 5.0 kGy. In the second-year cultivation season, red ripened peppers were harvested, dried in an oven (70°C). They were packaged into nylon sacks and vacuum sealed for γ-irradiation treatments with doses of 2.5 kGy, 7.5 kGy, and 10.0 kGy. In all, 2 kg of ripe red pods in triplicate from each cultivar were vacuum-sealed, irradiated, and analysed for phytochemicals each year. Unirradiated peppers in both seasons were used as control (0 kGy).

The γ-irradiation treatments were performed in the Isotope Institute of the Central Research Institute of Physics, Budapest, Hungary) using Cobalt 60 as a source of γ-irradiation. The γ-irradiation was performed at a rate of 1 kGy per hour for 0, 0.5, and 5 hours in the first season to achieve its final doses and; in the second season, 0, 2.5, 7.5, and 10 hours to achieve the final doses of 0, 2.5, 7.5, and 10 kGy respectively. After γ-irradiation, the pods were stored at ambient temperature for one week to over-ripen. The over-ripened pods were dried at 70°C for 24 hours using a drying cabinet with air circulation followed by milling in a coffee mill and passing through a 20-mesh sieve to obtain a uniform fine powder. The powders were stored at -20°C when not immediately analyzed.

3.5.1 Determination of capsaicinoids, carotenoids, and tocopherols in dried/ irradiated peppers

Capsaicinoids- 0.5 grams of powdered pepper was weighed in an Erlenmeyer flask and diluted in a 50 mL methanol (analytical grade). The mixture was ultrasonicated for 2 minutes and further shaken with a digital orbital shaker for 10 mins. The mixture was filtered using filter paper and diluted. 10 times (9:1) dilution process of 9 mL chromatography grade methanol: 1 mL filtrate for 'Hetényi Parázs' and 'Unikal' was purified through a 0.22 μm PTFE (Chromfilter) syringe into vails. 'Unijol' was subjected to 20 times (9:1,1:1) dilution process of 9 mL chromatography grade methanol: 1 mL filtrate and filtered through a 25 μm Chromfilter (syringe) into a 10 mL glass

beaker. The filtrate was further diluted using an Eppendorf pipette of 1 mL methanol and 1 mL filtrate (from syringe filter) into vails and injected into an HPLC column.

Carotenoids and Tocopherols- 0.5 grams of powdered was weighed in an Erlenmeyer flask and diluted in a 50 mL methanol-acetone solvent (2:1:1 prepared with 1000 mL dichloroethane: 500 mL methanol: 250 mL acetone). The mixture was ultrasonicated for 2 minutes and further shaken with a digital orbital shaker for 10 mins. The mixture was filtered using filter paper into a flat bottom flask and loaded in a rotary evaporation chamber for 10mins at 70°C and 40°C vacuum, respectively. The flask was offloaded from the chamber tube after all filtrate evaporated. Using an Eppendorf pipette, 5 mL of pigment eluents and 5 mL of methanol (liquid chromatography grade) were respectively dropped into the flask and shaken evenly. An ultrasonic shaker was used where necessary to ensure that no residue is left in the flask. The filtrate was further passed through a 0.22 μm PTFE membrane syringe filter into vails and injected into the HPLC column.

3.6 HPLC Instrumentation and chemical used

In all phytonutrient analyses, an HPLC (Hitachi Chromaster) instrument consisting of a Model 5110 Pump, a Model 5210 Auto Sampler, a Model 5430 Diode Array detector, and a Model 5440 FL detector, was used for the determination of all compounds. All analytical grade solvents and chemicals, as well as 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 $FeCl_3$ followed by reduction with $NaBH_4$ in ethanol according to Kruk et al. (2008).

3.7 Statistical analysis

Data were expressed as the mean ± standard deviation (SD) among physiological responses, pepper cultivars, water supply 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 water supply (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), water supply (0%, 50%, and 100%) and harvest periods (1^{st} harvest, 2^{nd} harvest, 3^{rd} harvest and 4^{th} 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.

4.0 RESULTS AND THEIR DISCUSSION

4.1 Effect of water supply treatments on physiological factors and cultivars during the growth period

During the 2018 and 2019 cultivation period, the various cultivars (HET, UNIK, UNIJ, and HAB) were subjected to three different water supply treatments (0% or control, 50% water deficit, and 100% optimum water supply) before measurement of physiological factors was done every two (2) weeks the first year and every week in the second year respectively.

4.1.1 Relative Chlorophyll content (expressed as SPAD values)

During the 2018 and 2019 growth period, the chili pepper cultivars (HET, UNIK, UNIJ, and HAB) were given different water supply treatments (0%, 50%, and 100%), and physiological parameters were measured. In the first growing season (2018), plant stands received more water due to rain (varied between 348 – 620 mm) when compared to the 2019 growing season (varied between 133 – 422 mm), which was mildly dry all through (fig. 8) in agreement with others (Goto et al., 2021).

In 2018 (fig. 9A), water supply had no significant influence on HET and UNIK even though a slight decrease in SPAD values was recorded in 50% and 100%. In UNIJ, lower SPAD values were recorded in 100% when compared to 0%; however, 100% was not significantly different from 50% ($F=6.687$, $p=0.002$) (fig. 9A). HAB had the lowest SPAD values significantly among all cultivars ($F=35.357$, $p<0.001$). UNIK recorded the highest relative chlorophyll content in all cultivars but was not significantly different from HET.

Similarly, all cultivars in 2019 (fig. 9B) had significant differences among them. There was no significant effect on water supply 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 water supply increased, SPAD content decreased in UNIJ. Also, in HAB, a decrease in SPAD values as the water supply 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%.

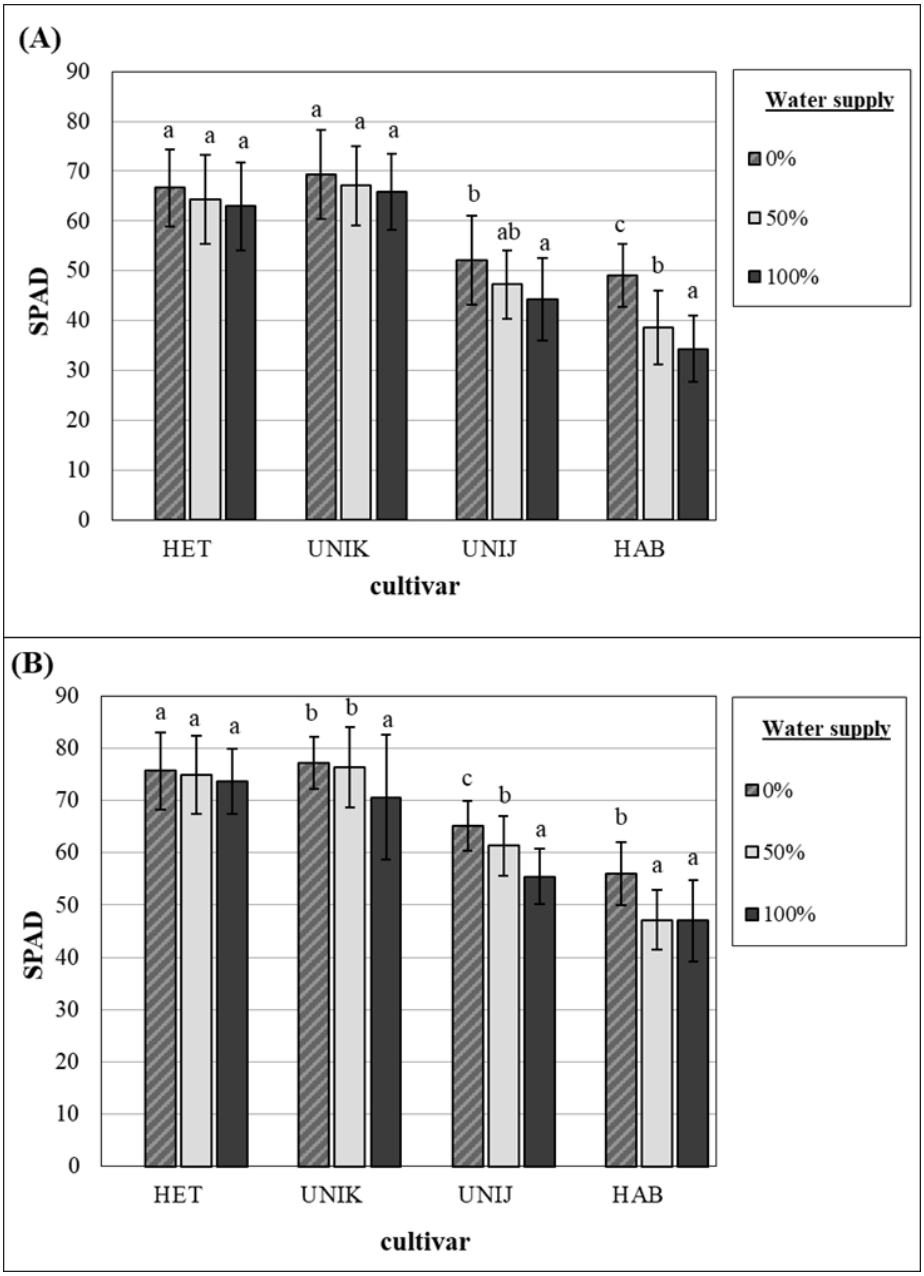


Figure 9. Effect of seasonal water supply treatments and cultivar response to relative chlorophyll content expressed as SPAD values in 2018 **(A)** and 2019 **(B).** 0%, control; 50%, deficit irrigation; 100%, optimum water supply; HET, HeténYi Parázs; UNIK, Unikal; UNIJ, Unijol; HAB, Habanero.

In both cultivation years, UNIK and HET pepper cultivars had higher relative chlorophyll content when compared to UNIJ and HAB. Peppers that were given no irrigation treatment (0%) had higher levels of chlorophyll content and the lowest in 100% optimum water supply. A measure

of relative chlorophyll content in plant leaves by SPAD-502 indicates photosynthetic productivity and the plant response to growth (Alonso et al., 2002; Jifon et al., 2005).

During the pepper plants' growth period, a decrease in relative chlorophyll content was observed in UNIJ and HAB (fig 9). Leaf surface area proves a reduction in meristematic cell activity in plant growth when there is a decrease in nitrogen supply (Rufty et al., 1988; Xiong et al., 2015). A decrease in cell expansion, which is also a contributing factor to relative chlorophyll content, was observed in previous studies of cotton and pepper plant leaves (Radin & Parker, 1979; Xiong et al., 2015).

4.1.2 Canopy temperature

Determining leaf surface temperature is one of the best indirect methods for determining the water supply of plants and scheduling irrigation (Jones, 1990). At the Department of Horticulture in Gödöllő, an infrared remote thermometer was used for the first time in Hungary to determine leaf surface temperature by this non-destructive way in the case of vegetable plants (Helyes and Varga, 1990).

Even though lower canopy temperatures were detected under 100%, water supply treatments had no effect on all cultivars during the 2018 growing season (fig 10A). Nevertheless, a gradual decrease in canopy temperature was recorded in cultivars as the water supply increased. HAB had a higher canopy temperature; however, they were not different from the other cultivars. UNIK had the lowest canopy temperature in 100% when compared to 50% and 0% ($F=1.687$, $p=0.192$).

On the effect of water supply treatments, in the 2019 season (fig 10B), HET had a significantly ($p=0.001$) lower response to canopy temperature under 50% and 100% conditions when compared to 0%. However, between 0% and 100%, there were no significant differences ($F=4.116$, $p=0.020$). Water supply had no influence on UNIK, UNIJ, and HAB. Notwithstanding, as water supply increased, canopy temperature increased in UNIJ, but on the contrary, that of HAB decreased as water supply increased even though there were no significant differences in them.

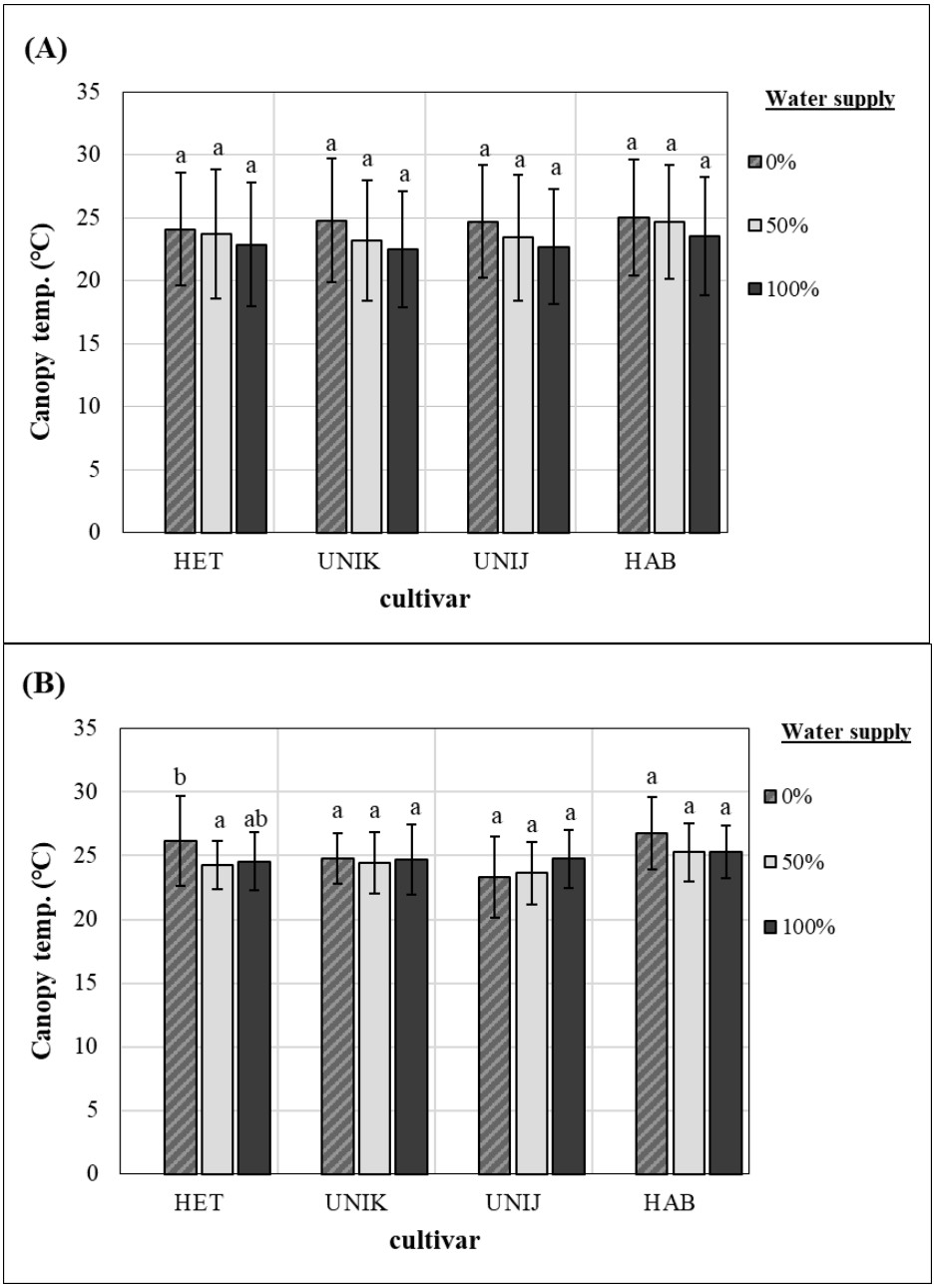


Figure 10. Effect of seasonal water supply treatments and cultivars' response to canopy temperature under unirrigated and irrigated conditions in 2018 **(A)** and 2019 **(B)**. 0%, control; 50%, deficit irrigation; 100%, optimum water supply; HET, Hetényi Parázs; UNIK, Unikal; UNIJ, Unijol; HAB, Habanero.

Canopy temperature measures the resistance of environmental stress conditions exposed to plants. The sensitivity of leaf stomata to water and carbon dioxide is necessary to leaf response to temperature (Morison, 1985). It was observed that there were no significant differences in cultivars planted (fig 10) over the growing period in the year 2018. However, the temperature was lower in

0% UNIJ peppers in the second year even though they were not significantly different from 50% and 100%. Plants that grow under elevated temperature result in elevated carbon dioxide, which influences freeze-resistance crops to frost damage (Barker et al., 2005; Loveys et al., 2006).

4.1.3 Chlorophyll fluorescence

The use of chlorophyll fluorescence measurements to study the photosynthetic performance and stress responses of algae and plants is widespread in ecophysiological studies; photosynthetic activity can be measured indirectly by chlorophyll fluorescence induction (Krause & Jahns, 2004).

The Fv/Fm in 0% were lower during the 2018 growth period (fig. 11A) even though an increase in water supply (50% and 100%) showed no significance in their values. Fv/Fm values between HET, UNIK, and UNIJ cultivars at each water supply level were in the same mean range. In the HAB cultivar, lower values were recorded in 0%. However, between 50% and 100%, Fv/Fm values were not significantly ($F=0.370$, $p=0.692$) different from each other. Water supply treatments did not influence Fv/Fm values in the chili cultivars, even though a lower response was detected in HAB.

In the second growing season (fig. 11B), HET had significantly ($p=0.021$) lower Fv/Fm values in 100%. However, there was no significant difference between 100% and 50% ($F=10.101$, $p<0.001$). Among the other cultivars (UNIK, UNIJ, and HAB), Fv/Fm values of water supply treatments were not significantly different from each other. Nonetheless, it was detected that as the water supply in HAB increased, Fv/Fm values decreased even though there were no significant differences among them ($F=2.537$, $p=0.085$).

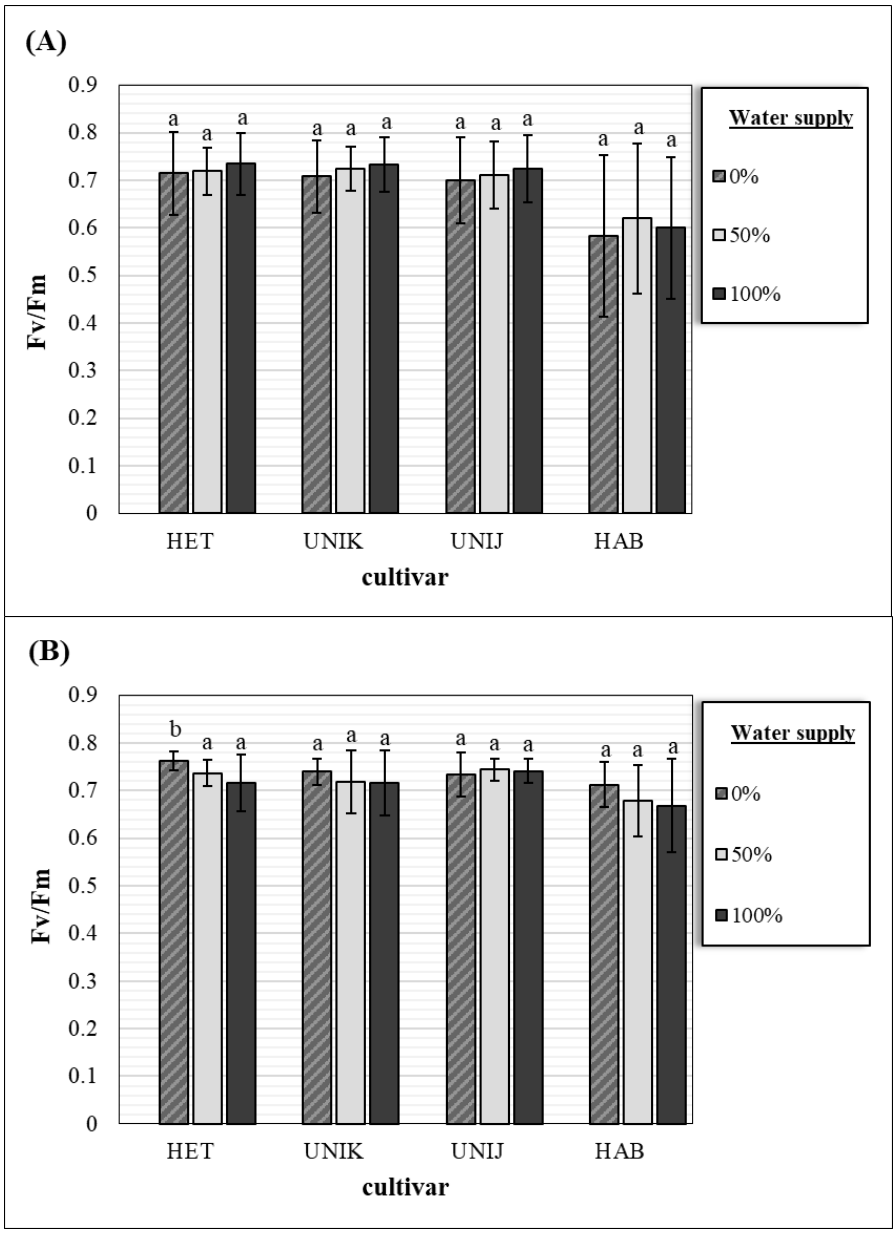


Figure 11. Effect of seasonal water supply treatments and cultivars' response to chlorophyll fluorescence (Fv/Fm) in 2018 **(A)** and 2019 **(B).** 0%, control; 50%, deficit irrigation; 100%, optimum water supply; HET, HeténYi Parázs; UNIK, Unikal; UNIJ, Unijol; HAB, Habanero.

In 2018 and 2019, 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).

4.1.4 Soil Moisture

Soil moisture contents corresponding to the actual phenological stages can be used to schedule irrigation and reduce water loss to sustain chilli pepper production in arid areas (Sharma et al., 2017).

During the 2018 growth season (fig. 12A), HET had significantly ($p=0.009$) lower soil moisture content under 0% when compared to 50% and 100%. A significant trend was also observed in 50% and subsequent in 100% ($F=24.653$, $p<0.001$). Similarly, in UNIK, significantly lower moisture content was recorded in 0% when compared to 50% and 100% ($F=30.507$, $p<0.001$). As water supply increased, soil moisture content increased as well in UNIJ. A significantly ($p=0.001$) higher moisture content was recorded in 100% and in 50% when compared to 0% ($F=36.854$, $p<0.001$). In HAB, between 50% and 100%, moisture content was not significantly different from each other; however, 0% had significantly lower soil moisture content ($F=30.334$, $p<0.001$).

Since the 2019 growing season (fig. 12B) 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% ($F=32.952$, $p<0.001$). UNIK had a similar trend to that of HET as soil moisture between 50%, and 100% was significantly higher when compared to 0% ($F=25.372$, $p<0.001$). The same trend was observed in UNIJ ($F=29.634$, $p<0.001$) and HAB ($F=45.835$, $p<0.001$). 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.

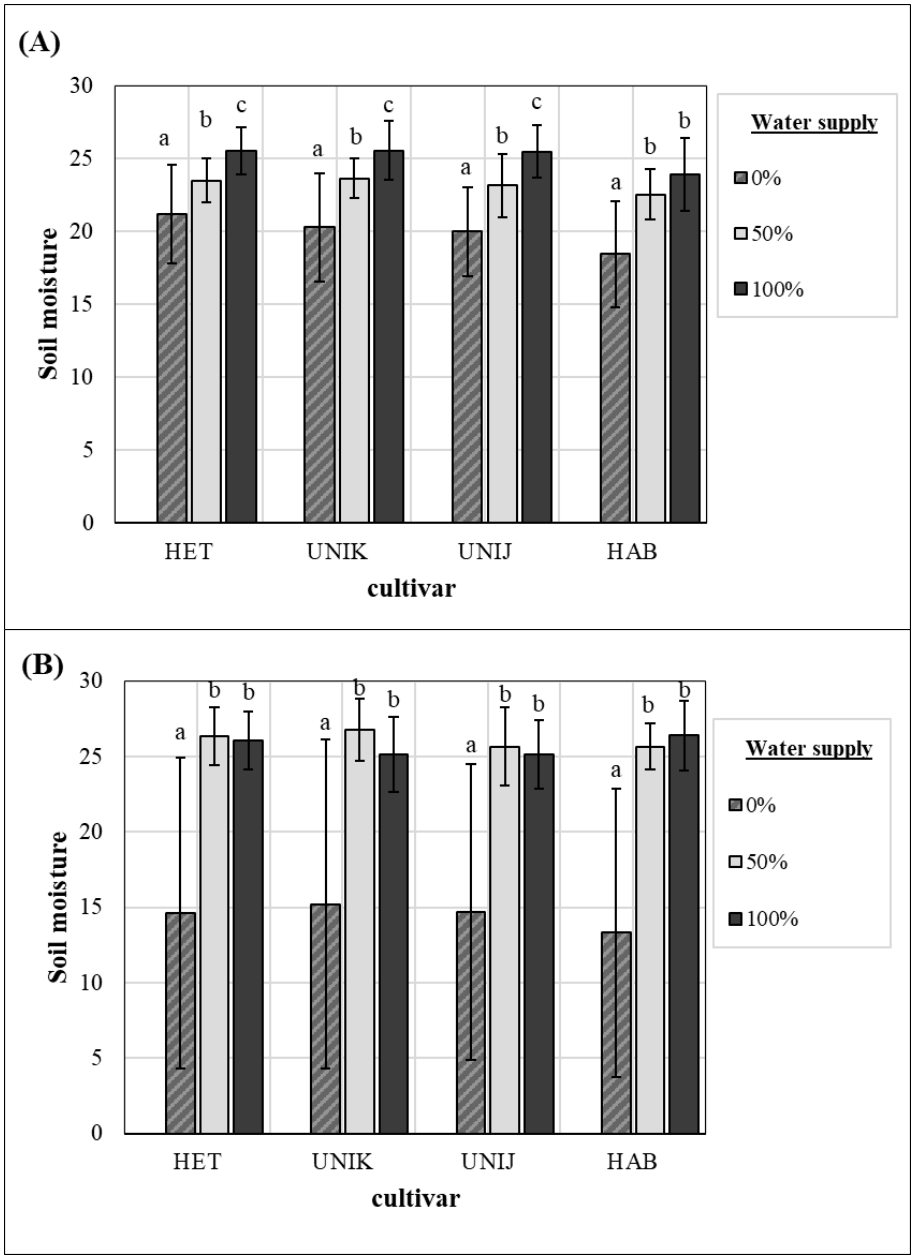


Figure 12. Effect of seasonal water supply on the cultivars planted and their response to soil moisture in 2018 **(A)** and 2019 **(B).**

Peppers cultivated under the various water supply treatments had a significant influence ($p<0.05$) in the first year and also in the second growing season (fig. 12). The use of an alternate drip irrigation system can improve yield in hot pepper cultivation, especially in areas where irrigation is necessary for crop management practices (Kang et al., 2001). The effect of moisture during the first year of cultivation showed that higher moisture in soil affects crop development which

corroborates a study conducted by (Fawusi, 1978) on sweet pepper seed emergence. The use of regulated irrigation and soil moisture was used to control blossom-end rot in hot peppers (Dorji et al., 2005).

4.2 Effect of water supply on yield of cultivars

The weight of the fruits was measured, and the yield per hectare was calculated. During the harvesting period in the 2018 cultivation year (Table 3), UNIJ recorded a significantly lower yield when compared to UNIK and HET. HAB was still at its fruiting stage and was not ready during the first harvest. Between the water supply treatments, HET recorded a significantly higher yield in the second harvest (50%) when compared to the 0% and 100%. A decline in yield was observed in the third harvest and subsequently in the fourth harvest.

UNIK recorded a higher yield in the second harvest when compared to the first, third, and fourth harvests. UNIK peppers by the fourth harvest had no fruits. However, between the water supply treatments, higher yields were produced under 50% conditions. Similarly, UNIJ recorded lower yield in the first harvest and higher yields in the second harvest (50%) but declined in the third harvest and subsequently in the fourth harvest. HAB, on the other hand, recorded a lower yield in the second harvest and increased in the third harvest, and reduced by the fourth harvest. However, comparing the effect of water supply treatments, HAB peppers cultivated under 100% conditions had significantly ($p<0.05$) lower yield.

Between the cultivars, HET recorded averagely higher yields when compared to UNIK, UNIJ, and HAB.

Table 3: The average yield of the peppers cultivated in 2018 (n = 4; mean ± SD) based on fresh weight (t/ha)

Cultivar	Water supply treatments	1st harvest	2nd harvest	3rd harvest	4th harvest	Total yield (t/ha)
HET	0%	3.55±0.86Ba	10.39±2.27Ca	2.31±0.57Ba	0.36±0.07Aa	16.62±0.94a
	50%	3.6 ± 0.72Ba	11.95 ± 3.23Ca	2.43 ± 0.45Ba	0.36 ± 0.03Aa	18.34±1.11b
	100%	3.17±0.77Ba	10.39±2.36Ca	1.72±0.37Aa	0.36±0.10Aa	15.64±0.90a
UNIK	0%	1.86±0.37Aa	9.86±1.76Ca	4.18±1.33Ba	-	15.9±1.16a
	50%	2.05 ± 0.47Aa	10.52 ± 1.91Ca	7.1 ± 1.28Bb	-	19.67±1.22b
	100%	1.97±0.42Aa	9.02±2.07Ca	4.26±0.81Ba	-	15.25±1.10a
UNIJ	0%	0.85 ± 0.24Aa	6.64 ± 1.55Ca	2.58 ± 0.17Ba	0.36 ± 0.03Aa	10.43±0.50a
	50%	0.92 ± 0.30Aa	10.02 ± 3.31Bb	1.62 ± 0.34Aa	0.24 ± 0.03Aa	12.8±1.00b
	100%	0.52±0.10Aa	6.89±1.84a	1.26±0.20Aa	0.24±0.03Aa	8.92±0.54a
HAB	0%	1.81±0.55Ab	5.63±3.32Bb	0.48±0.24Aa		7.92±0.79b
	50%	2.19±1.25Bb	5.41±1.96Cb	0.36±0.07Aa		7.96±1.09b
	100%	0.55±0.20Aa	3.32±0.63Ba	0.24±0.06Aa		4.11±0.30a

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

During the 2019 growing season (Table 4), HET had the highest yield during the first harvest (0%) and significantly reduced in the second harvest, increased by the third harvest and further decreased by the fourth harvest. Under deficit irrigated conditions (50%), the yield of HET reduced after the first harvest in the subsequent harvesting periods. UNIK recorded higher yields under 50% but were not significantly different from 100%. Between the various harvesting periods, UNIK recorded higher yields in the first harvest when compared to the second, third, and fourth harvests. It was also observed that yield reduces as the continuous harvest is done.

UNIJ, on the other hand, recorded higher yields in the second and third harvest when compared to the first and fourth harvests. Between the water supply treatments, UNIJ peppers produced a higher yield under 50% irrigated conditions when compared to the 0% and 100%. Similar to the 2018 growing season, HAB was not ready during the first harvesting period. Between the harvesting periods, the yield of HAB was significantly higher ($p<0.05$) in the third harvest (100% optimum water supply) when compared to the second and fourth harvests.

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

Cultivar	Water supply treatments	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

The study results in the first year of cultivation (Table 3) indicated higher yields (t/ha) from deficit-irrigated pepper plants in the second harvest. Generally, the total yield harvest of irrigated peppers increased (HET, 18.34±1.11 t/ha; UNIK, 19.67±1.22 t/ha; UNIJ, 12.8±1.00 t/ha) when compared to 0% and 100%. Sezen et al. (2014) reported higher yield and fruit quality in red peppers cultivated in the Mediterranean region. Under optimum water supplied conditions, a lower yield was observed in HAB (4.11±0.30 t/ha). Previous studies indicated that moderate and extreme climates might affect the growth and yield of hot peppers (Lee et al., 2018). A decrease in yield in hot peppers (HAB) under less water supply may be due to poor flowering and fruiting (Jaimez et al., 1999). A decline in yield by the fourth harvest, as observed in this study, could be attributed to the uncontrolled conditions and beginning of the frost or winter period due to exposure of plants to the environment (Juroszek & Tsai, 2009). Studies have indicated that greenhouse cultivated hot peppers responded well to growth and yield (Guang-Cheng et al., 2010).

Compared to the second year growing season (Table 4), the total average yield of HET (24.67 ± 3.34 t/ha) increased under non-irrigation and 50%. The total yield of UNIJ under non-irrigated (3.76 ± 0.77 t/ha) reduced when irrigated (20.45 ± 3.89 t/ha) and further in HAB under

deficit-irrigated conditions (3.36±0.47 t/ha). Continuous water-stressed *Capsicum* species may affect fresh fruit weight and yields (Dalla Costa & Gianquinto, 2002; Gençoğlan et al., 2006). Photosynthetic activities are influenced by light intensity, leaf position, and plant growth stage (Dong et al., 2014). According to the literature, physiological responses can subsequently have an impact on the yield value of peppers. Therefore inadequate photosynthetic activity and water stress reduced about 30% yield in sweet peppers cultivated under open field (Delfine et al., 2001).

4.3 Determination of phytochemicals in fresh peppers

4.3.1 Total capsaicinoids concentration

The peppers were subjected to different water supply treatments for two consecutive growing seasons to study their pungency changes. A significant increase ($p<0.05$) in NDC, CAP, DC, HCAP, iDC, and HDCs was observed in the year 2018 (Table 5).

In the HET cultivar, water supply treatments had no influence on NDC concentration in the first and second harvest periods. However, in the third harvest, NDC was higher in the 0% and significantly ($p=0.021$) lower in 50% and 100%. Also, in the fourth harvest, NDC was significantly ($p=0.014$) lower in 100% when compared to 0% and 50%. Between 0% and 50%, no significant differences were found. In UNIK, water supply did not influence NDC concentration in the first and second harvests. In the third harvest, concentration was significantly ($p=0.009$) lower in the 50% when compared to 0%, even though concentration in both 50% and 100% were not significantly ($p=0.069$) different from each other. NDC concentration in the fourth harvest was significantly ($p=0.015$) higher in 100% when compared to 0%; however, under 50% deficit, concentration was not different from 0% and 100%.

In UNIJ, water supply did not have any influence on NDC concentration in the first, second and third harvest periods. However, in the fourth harvest, concentration was found to be significantly ($p=0.005$) higher in 50% and 100%. Between 50% and 100%, no differences were found. In HAB, water supply treatments did not have an influence on the first and second harvests. However, in the third harvest, NDC concentration was found to be significantly ($p=0.001$) lower in 50% and 100% when compared to 0%.

For the level of CAP in the HET cultivar, water supply treatments had no influence on their concentration in the first, second and third harvests. However, in the fourth harvest, concentration was found to be significantly ($p=0.003$) lower in 100% when compared to 0% and 50%. Between 0% and 50% concentration were not different from each other. In UNIK, water supply had no

influence on CAP concentration in the first harvest, but in the second harvest, amounts were significantly ($p=0.015$) higher in the 50% and 100% when compared to 0%. Also, in the third harvest, CAP concentration was significantly ($p=0.044$) lower in 50% when compared to 0%. In the fourth harvest, concentration was significantly ($p=0.006$) higher in 100% when compared to 0% and 50%.

In UNIJ, water supply did not affect CAP concentration in the first, second and third harvests. However, in the fourth harvest, concentration was found to be significantly ($p=0.001$) higher in 50% and 100% even though between 50% and 100%, concentration did not differ. In HAB, water supply had no influence on CAP concentration in the first and second harvests. However, in the fourth harvest, significantly ($p\leq0.001$) lower amounts of CAP were found in 100% when compared to 0% and 50%.

Water supply had no influence on DC concentration in HET in the first, second and third harvest periods. However, in the fourth harvest, concentration was found to be significantly ($p=0.007$) lower in 100% when compared to 0% and 50%. Between 0% and 50%, concentration did not change. In UNIK, water supply did not affect DC concentration in the first and second harvests, but in the third harvest, concentration ($p=0.004$) lower by 50% and 100% when compared to 0%. Also, in the fourth harvest, as the water supply increased, DC concentration increased significantly ($p=0.017$) in 100% when compared to 0%. In UNIJ, water supply did not influence DC in the first, second and third harvests, but in the fourth harvest, concentration significantly ($p=0.004$) increased in 50% and 100% when compared to 0%. In HAB, water supply did not affect DC concentration in the first harvest. In the second harvest, concentration was significantly ($p=0.051$) lower in 50% when compared to 0%, even though concentration did not change in 100%. Also, in the third harvest, as the water supply increased, concentration significantly ($p\leq0.001$) decreased.

In HET, water supply did not have any effect on HCAP concentration in all the harvests. Water supply had no influence on HCAP concentration in the third harvest. In HAB, significantly ($p=0.001$) lower amounts were found in 50% and 100% when compared to 0%.

On the amount of iDC, the water supply in HET was not affected in the first and second harvests. However, in the third harvest, concentration was significantly ($p=0.004$) lower in 50% and 100%. Also, in the fourth harvest, concentration was significantly ($p=0.015$) lower in the 100% optimum water when compared to 0%. In UNIK, water supply had no influence on iDC concentration in the first, second and fourth harvests. However, in the third harvest, concentration was significantly

(p=0.013) lower in 50% and in 100%. Similarly, in UNIJ, water supply did not affect concentration in the first, second and fourth harvests. Nevertheless, in the third harvest, significantly (p=0.013) higher amounts were found in the 50% and 100% when compared to 0%. In HAB, water supply had no influence on iDC concentration in all harvest periods.

Water supply had no effect on HDCs in HET in the first and second harvests, but in the third harvest, a significantly (p=0.018) lower concentration were found in 100% when compared to 0%, even though no change was recorded in 50%. Also, in the fourth harvest, HDCs was significantly (p=0.017) lower in 100% when compared to 0% and 50%. Water supply had no influence on HDCs in UNIK in the first, second and third harvests. However, in the fourth harvest, significantly (p=0.030) higher amounts were found in 100% when compared to 0%. A similar trend was recorded in UNIJ even though by the fourth harvest, HDCs were significantly (p=0.001) higher in 50% and 100% when compared to 0%. In HAB, water supply had no effect on concentration in the first and second harvests. Nonetheless, in the third harvest, concentration was significantly (p=0.001) lower in 50% and 100% when compared to 0%.

Table 5: Effect of water supply on capsaicinoid concentration in the various red pepper cultivars and harvesting periods for the 2018 growing season. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Capsaicinoid	Water supply treatments/ Cultivar	1st harvest	2nd harvest	3rd harvest	4th harvest
NDC					
	HET				
	0%	95.4±123.1Aa	9.6±2.5Aa	44.4±6.1Ab	36.2±6.2Ab
	50%	33.2±3.1Ba	9.1±1.8Aa	30.4±4.4Ba	33.8±6.7Bb
	100%	28.3±3.5Ba	8.9±1.9Aa	29.9±8.7Aa	21.8±4.1Aa
	UNIK				
	0%	18.5±3.5Ba	2.8±0.0Aa	22.2±4.3Ab	16.6±2.3Aa
	50%	16.1±3.7Aa	4.0±1.3Aa	10.4±2.4Aa	26.8±21.5Aab
	100%	15.7±2.1Aa	3.7±1.3Aa	14.2±5.2Aab	55.3±15.0Bb
	UNIJ				
	0%	608.1±87.3Ca	116.4±11.6ABa	204.7±20.3Ba	84.0±11.4Aa
	50%	566.1±126.1Ba	117.2±9.3Aa	181.1±30.3Aa	229.2±23.6Ab
	100%	582.1±141.3Ba	109.4±20.9Aa	185.5±24.4Aa	242.4±91.3Ab
	HAB				
	0%	62.1±13.5Ba	47.2±10.9Ba	184.6±9.2Cc	
	50%	70.9±14.1Ba	24.0±16.2Aa	140.0±12.4Cb	
	100%	72.6±23.4Ca	33.2±6.7Ba	111.1±16.0Da	
CAP					
	HET				
	0%	310.1±46.3Ca	55.2±15.5Aa	238.0±34.8Ba	344.9±22.9Cb
	50%	269.6±14.9Ba	66.7±11.6Aa	234.5±21.8Ba	370.4±55.0Cb
	100%	264.0±30.4Ba	61.1±12.2Aa	252.5±51.3Ba	221.0±55.4Ba
	UNIK				
	0%	94.3±20.6Ba	17.3±1.7Aa	78.7±13.7Ba	85.4±20.8Ba
	50%	86.2±6.0Aa	29.2±7.9Ab	50.2±5.9Aa	201.7±208.1Aa
	100%	100.9±18.4Aa	30.3±4.9Ab	67.2±18.1Aab	507.6±126.8Bb
	UNIJ				
	0%	6173.1±562.4Da	1156.7±187.3Ba	2598.7±225.7Ca	427.0±103.9Aa
	50%	6518.7±764.5Ca	1239.9±281.2Aa	2383.5±314.7Ba	2081.6±467.9ABb
	100%	5472.8±1140.0Ba	1213.6±153.3Aa	2417.6±468.3Aa	2282.8±768.3Ab
	HAB				
	0%	1903.1±362.3Ca	1258.2±328.1Ba	3564.7±150.2Db	
	50%	2191.8±355.1Ca	729.7±537.3Ba	3211.2±85.7Db	
	100%	2130.6±376.5Ca	1029.0±234.0Ba	2730.0±259.9Da	
DC					
	HET				
	0%	158.2±18.7Ba	39.4±9.8Aa	192.5±16.7Ca	189.8±13.8BCb
	50%	149.9±10.5Ba	43.7±7.2Aa	162.7±18.4Ba	196.0±39.1Bb
	100%	135.9±14.4Ba	41.1±7.4Aa	151.0±27.4Ba	117.0±29.2Ba
	UNIK				
	0%	62.1±6.9Ba	13.5±2.4Aa	78.9±11.6Bb	72.8±13.2Ba
	50%	59.1±5.1Aa	19.8±5.6Aa	43.2±9.7Aa	140.5±135.0Aab
	100%	62.8±8.4Aa	18.5±3.6Aa	55.0±12.1Aa	302.5±82.3Bb
	UNIJ				
	0%	3556.8±482.7Ca	796.2±36.9Aa	1592.5±95.2Ba	364.0±65.9Aa
	50%	3635.6±379.5Ca	766.5±61.1Aa	1404.3±253.7Ba	1261.7±300.0ABb
	100%	3330.8±815.1Ba	744.6±71.4Aa	1355.3±200.8Aa	1331.7±486.5Ab
	HAB				
	0%	967.7±218.6Ca	601.1±135.1Bb	2131.5±218.7Dc	

	50%	1080.6±209.2Ba	292.2±212.2Aa	1576.7±133.0Cb	
	100%	1029.8±189.1Ca	399.8±81.6Bab	1098.1±145.9Ca	
HCAP					
	HET				
	0%	8.7±3.1Ba	1.17±0.3Aa	5.1±0.8ABa	5.2±3.2ABa
	50%	6.2±0.9Ca	1.24±0.3Aa	1.2±0.3Ba	6.4±0.8Ca
	100%	5.2±1.2Ba	1.2±0.3Aa	4.2±1.2Ba	4.2±2.3Ba
	UNIK				
	0%	ND	ND	ND	ND
	50%	ND	ND	ND	ND
	100%	ND	ND	ND	ND
	UNIJ				
	0%	ND	ND	ND	ND
	50%	ND	ND	ND	0.7±1.6Aa
	100%	ND	ND	ND	ND
	HAB				
	0%	ND	ND	30.8±5.0Bb	
	50%	ND	ND	21.1±2.3Ba	
	100%	ND	ND	15.6±2.6Ba	
iDC					
	HET				
	0%	9.9±4.3ABa	4.1±1.3Aa	19.0±3.5Cb	12.0±3.0Bb
	50%	14.2±2.4Ca	3.5±0.6Aa	9.8±1.8Ba	9.7±2.5Bab
	100%	11.7±1.8Ca	3.7±0.7Aa	9.5±4.0BCa	6.1±0.4ABa
	UNIK				
	0%	5.4±3.0Ba	1.05±0.4Aa	5.7±1.9Bb	4.3±1.3ABa
	50%	3.8±1.7Aa	1.6±0.3Aa	3.8±1.7Aa	3.1±1.4Aa
	100%	4.7±0.9Ca	0.9±0.3Aa	4.7±0.9Ba	4.5±0.9BCa
	UNIJ				
	0%	241.5±23.5Ba	41.1±10.0Aa	28.8±9.6Ab	52.5±10.3Aa
	50%	218.7±61.4Ba	44.62±11.9Aa	11.4±5.1Aa	70.8±5.2Aa
	100%	271.8±71.8Ba	35.0±5.7Aa	14.8±4.4Aa	83.1±31.6Aa
	HAB				
	0%	42.0±9.9Ba	18.3±7.8ABa	84.7±21.1Ca	
	50%	42.8±9.2Ba	11.3±8.7Aa	70.7±6.6Ca	
	100%	54.2±25.0Ba	20.1±3.3Aa	53.4±16.3Ba	
HDCs					
	HET				
	0%	15.3±1.5Ba	5.3±2.3Aa	21.3±2.3Cb	17.1±1.9Bb
	50%	15.7±1.4Ba	4.2±1.0Aa	16.5±1.0Bab	16.7±4.1Bb
	100%	13.4±1.9Ba	4.1±1.1Aa	15.5±3.8Ba	10.7±2.2Ba
	UNIK				
	0%	6.3±1.0Ba	1.0±0.4Aa	8.63±1.5Ca	6.6±0.9Ba
	50%	5.7±0.9ABa	1.6±0.7Aa	6.6±1.7ABa	8.5±4.6Bab
	100%	5.9±0.9ABa	1.7±0.4Aa	6.7±1.7Ba	14.3±3.7Cb
	UNIJ				
	0%	177.7±21.0Ca	35.0±6.1Aa	68.5±12.1Ba	9.6±12.2Aa
	50%	178.5±36.0Ba	35.0±15.1Aa	65.5±13.5Aa	59.5±7.6Ab
	100%	173.8±39.1Ba	33.8±9.0Aa	89.1±55.6ABa	63.8±21.6Ab
	HAB				
	0%	22.7±4.9Ba	17.9±6.1Ba	49.8±4.4Cb	
	50%	24.4±5.5Ba	9.2±6.6ABa	21.0±20.2ABa	
	100%	25.3±7.8Ca	12.3±2.0Ba	2.6±1.7Aa	

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; ND: not detected; NDC: nordihydrocapsaicin; CAP: capsaicin; DC: dihydrocapsaicin; HCAP: homocapsaicin; iDC: dihydrocapsaicin isomer; HDCs: homodihydrocapsaicins.

A significant increase ($p<0.05$) in NDC, CAP, DC, HCAP, iDC, and HDCs was observed in the harvesting periods and water supply treatments in the 2019 season (Table 6).

In HET, the effect of water supply on NDC concentration in the first harvest was found to be significantly ($p\leq0.001$) lower in 50% and 100%. It was continuously detected in the second ($p=0.006$), third ($p=0.002$), and fourth ($p\leq0.001$) harvests under the same water supply conditions. In UNIK, water supply treatments had no significant ($p>0.05$) effect on NDC concentration in the first, second and third harvests. However, in the fourth harvest, concentration was significantly ($p=0.006$) lower in 50% and 100%. In UNIJ, water supply had no significant ($p=0.187$) influence on NDC concentration in the first harvest. In the second harvest, concentration was found to be significantly ($p\leq0.001$) lower in 50% and 100%, and also, similarly in the third harvest ($p\leq0.001$) under the same water supply conditions. Water supply was significantly ($p=0.024$) lower under 50% deficit in the fourth harvest when compared to 0% even though between 0% and 100%, no differences was recorded. In HAB, concentration was significantly ($p\leq0.001$) lower in 50% and 100% in the first harvest when compared to 0%. NDC concentration significantly ($p\leq0.001$) lowered in 50% and 100% in the second harvest. Also, concentration lowered significantly ($p\leq0.001$) in 50% and 100% when compared to 0% under the fourth harvest.

CAP concentration in HET was found to be significantly ($p=0.038$) lower in 50% under the first harvest when compared to 0%. However, between 0% and 100%, the concentration did not change. Water supply had no influence on CAP concentration in the second and third harvests. Concentration was significantly ($p=0.007$) lower in 50% when compared to 0% and 100% in the fourth harvests. In UNIK, CAP concentration was significantly ($p=0.022$) lower in 50% when compared to 0% in the first harvest. Water supply had no influence on concentration in the second, third and fourth harvests. In UNIJ, water supply had no influence on concentration in the first, third and fourth harvests. However, in the second harvest, a significantly ($p\leq0.001$) lower CAP was found in 50% and 100% when compared to 0%. In HAB, a significantly ($p=0.032$) higher CAP concentration was found in 50% and lower in 100% in the first harvest. Water supply had no effect on concentration in the second harvest. However, in the third harvest, a significantly ($p=0.037$) higher concentration was found in 50% and lower in 100% and 0%.

DC concentration in HET in the first harvest was found to be significantly ($p=0.001$) lower in 50% and 100% when compared to 0%. Also, concentration was significantly ($p=0.019$) lower in 100% and 50% in the second harvest even though between 50% and 0%, concentration did not

differ. Water supply had no influence on concentration in the third harvest, but in the fourth harvest, DC concentration was found to be significantly ($p=0.001$) lower in the 50% deficit when compared to 0% and 100% optimum water. In UNIK, water supply had a significant ($p=0.027$) effect on DC concentration in the first harvest. However, in the second, third and fourth harvests, water supply had no effect on concentration. In UNIJ, water supply had no influence on DC concentration in the first and third harvests. Nevertheless, concentration was found to be significantly lower in 50% and 100% of the second ($p\leq0.001$) and fourth ($p=0.002$) harvests. As the water supply increased, DC concentration in HAB decreased significantly ($p\leq0.001$) in the first harvest. Also, in the second harvest, concentration was found to be significantly ($p\leq0.001$) 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 between 50% and 0%, no change was detected.

In the minor or homologues, water supply had no effect on HCAP amounts in HET in both the first and second harvests. However, in the third harvest, HCAP was significantly ($p\leq0.001$) lower in 100% when compared to 50% and 0%. Also, in the fourth harvest, concentration was significantly ($p\leq0.001$) lower in 50% and 100% when compared to 0%. Water supply had no effect on HCAP amounts in UNIK and UNIJ in all harvest periods. In the case of HAB, significantly lower amounts were found in 50% and 100% in the second ($p\leq0.001$) and third ($p=0.010$) harvests when compared to 0%. Water supply had no effect on HCAP amounts in the fourth harvest.

In HET, iDC were significantly lower in 50% and 100% when compared to 0% in the first ($p=0.011$), second ($p\leq0.001$) and fourth ($p\leq0.001$) harvests. Also, in the third harvest, as the water supply increased, iDC significantly ($p\leq0.001$) decreased. Water supply had no influence on iDC amounts in UNIK in the first harvest. However, in the second ($p=0.008$), third ($p=0.004$) and fourth ($p=0.001$) harvests, iDC were significantly lower under 50% and 100% conditions. In UNIJ, water supply had no effect on iDC in the first harvest; however, in both the second ($p=0.012$) and third ($p=0.016$) harvests, iDC was significantly lower in 100% and 50% when compared to 0%, even though between 0% and 50%, the amount did not differ. A significantly ($p=0.004$) lower iDC were found in 50% and 100% in the fourth harvest when compared to 0%. In HAB, the iDC amount was significantly ($p=0.005$) lower in 50% and 100% in the first harvest. Also, in the second harvest, a significantly ($p=0.003$) lower iDC were found in 50% and 100% when compared to 0%. In the fourth harvest, amounts were significantly ($p=0.020$) higher in 100% when compared to 0% and 50%.

In the HET cultivar, HDCs were found to be significantly ($p \leq 0.001$) lower in 50% and 100% in the first harvest when compared to 0%. Also, in the second harvest, HDCs were significantly ($p=0.006$) lower in 100% and 50% when compared to 0% even though between 0% and 50%, amounts did not differ. A similar trend was observed in the third harvest; as the water supply increased, HDCs decreased significantly ($p=0.003$) in 100% and 50% even though between 0% and 50%, the amount did not change. In the fourth harvest, HDCs were significantly ($p \leq 0.001$) lower in 50% and 100% when compared to 0%. In UNIK, as the water supply increased, HDCs decreased significantly ($p=0.044$) in 50% and 100% when compared to 0%. However, in the second and third harvests, water supply had no influence on HDCs. In the fourth harvest, HDCs were significantly ($p=0.012$) lower in 50% and 100% even though between 0% and 100%, concentration did not change. In UNIJ, HDCs in the first harvest were significantly ($p=0.042$) higher in 50% when compared to 0%. However, between 50% and 100%, HDCs were the same. Water supply significantly ($p=0.007$) lowered HDCs in 100% when compared to 0% and 50% in the second harvest. In the third harvest, HDCs were significantly ($p \leq 0.001$) lower in 100% and 50%. Also, in the fourth harvest, HDCs significantly ($p=0.033$) decreased in 100% and 50% when compared to 0% even though between 50% and 0%, amounts did not differ. In HAB, HDCs were significantly lower in 100% and 50% deficit in the first ($p \leq 0.001$), second ($p \leq 0.001$) and third ($p \leq 0.001$) harvests when compared to 0%.

Table 6: Effect of water supply on capsaicinoid concentration in the various pepper cultivars for the 2019 growing season. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Capsaicinoid	Water supply treatments/ 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	151.4±21.6Ab	236.2±17.5Bb	350.0±56.3Cb
	50%	269.5±37.4Ba	95.4±10.4Aa	241.5±42.9Aa	241.5±42.9Ba
	100%	241.5±46.6Ba	77.0±14.6Aa	98.0±18.3Aa	288.7±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%	452.3±13.5Aa	1662.5±235.4Bb	1936.3±216.5Ba	2743.1±429.8Ca
	50%	498.7±21.8Aa	659.7±86.4Aa	1866.3±404.3Ba	2513.0±354.2Ca
	100%	1435.8±1919.7Aa	525.9±88.1Aa	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%	2072.0±170.6Ba	1108.6±156.8Ab	1418.3±126.6Aa	2195.3±244.7Bb
	50%	2920.7±567.1Ca	400.7±44.7Aa	1313.3±422.6Ba	1463.8±453.9Ba
	100%	2537.5±454.8Ca	313.2±55.5Aa	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	

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 water supply according to Tukey's HSD post hoc test; ND: not detected.

The main composition of capsaicinoids responsible for pungency is capsaicin and dihydrocapsaicin, as reported by the literature, were found in higher amounts as well, as nordihydrocapsaicin which is usually characterised as a homologue (Kurian & Starks, 2002), were also present in higher concentration in the cultivated peppers (HET, UNIK, UNIJ, and HAB).

A higher concentration of capsaicin (CAP) which contributes to about 60% of pungency in peppers (Topuz & Ozdemir, 2007), was evident in HET (310.1±46.3 µg/g) and HAB (3564.7±150.2 µg/g) when they were subjected to 0% and in UNIJ (6518.7±764.5 µg/g) under 50% (Table 5). Extreme water stress results in capsaicin's stability in hot peppers (Ruiz-Lau et al., 2011) but varies from cultivar (Phimchan et al., 2012). Lower levels of pungency were recorded in all cultivars when they were given optimum water supply and higher in non-irrigated and deficit irrigated peppers. This confirms a previous study by Jeeatid et al. (2018) that water stress influences higher pungency levels in hot peppers. It was also observed in UNIK peppers that NDC, CAP, and DC concentration decreased as water supply treatments increases. A general decrease in pungency as irrigation or water supply treatments increased was also evident in other pepper cultivars in this research. Studies have shown that changes in capsaicinoid concentration under the various water supply treatments are usually attributed to uncontrolled environmental conditions (Harvell & Bosland, 1997).

A similar trend was observed in the 2019 growing season, when capsaicin (CAP) concentration in HET (584.1±19.1 µg/g) and was higher under 0% in the first harvesting period and decreased as irrigation increased. Between the first harvest and fourth harvest, these major capsaicinoids compounds were significantly higher ($p<0.05$) in the first and fourth harvest (Table 6). Based on the results, the homologue compounds homocapsaicin, dihydrocapsaicin isomer, and homodihydrocapsaicins as displayed on the HPLC profile (Appendix 1) were found in smaller quantities in both years even though HCAP was absent in UNIK and UNIJ peppers due to changes in capsaicinoids accumulation behaviour in peppers (González-Zamora et al., 2013).

It was observed in this study that lower capsaicinoid concentration in UNIK peppers when compared to the other cultivars might be as a result of their inability to withstand climatic conditions, which contributes to a reduction in pungency in peppers (Gurung et al., 2011). Changes in pungency level in peppers in the 2018 and 2019 growing seasons, based on this study, varied between cultivars (Iqbal et al., 2013).

4.3.2 Vitamin C

The composition of vitamin C for both years was identified on the HPLC (Appendix 2).

In the 2018 season (Table 7), water supply treatments had no effect on vitamin C content in HET in the first, third and fourth harvests. However, in the second harvest, vitamin C content was significantly ($p=0.029$) lower in 100% and 50% when compared to 0% even though between 0% and 50%, the content did not differ.

Vitamin C content in UNIK significantly ($p=0.025$) decreased at 100% in the first harvest when compared to 0%. Between 0% and 50%, the content did not change. Water supply had no influence on vitamin C content in the second and fourth harvests. However, in the third harvest, vitamin C content was significantly ($p=0.002$) lower in 100% and 50% when compared to 0%.

Water supply had no influence on vitamin C content in UNIJ in the first harvest. In the second harvest, as the water supply increased, vitamin C content significantly ($p\leq0.001$) increased. Similar to the second harvest, as the water supply increased, vitamin C significantly ($p\leq0.001$) increased during the third harvest. Vitamin C decreased significantly ($p=0.022$) in 100% and 50% when compared to 0% in the fourth harvest. Water supply had no influence on HAB in all harvest periods.

Table 7: Effect of harvesting periods and water supply treatments on Vitamin C content in 2018 cultivation season. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

	Vitamin C 2018			
Water supply treatments/ Cultivar	**1st harvest**	**2nd harvest**	**3rd harvest**	**4th harvest**
HET				
0%	4161.0±439.2Ba	1132.5±67.2Ab	2196.6±227.9Aa	1820.9±366.4Aa
50%	3692.3±257.2Ca	983.3±40.8ABab	2229.4±93.4Ba	1289.9±724.3Aa
100%	3885.0±653.4Ca	901.5±55.8ABa	1988.9±244.4Ba	1090.6±326.5Aa
UNIK				
0%	3931.0±260.3Cb	827.8±53.6Aa	2439.7±216.1Bb	1896.5±243.8Aa
50%	3486.5±515.4Bab	735.9±37.7Aa	2025.7±114.5Aa	1539.6±282.4Aa
100%	3063.8±243.5Ca	733.6±26.4Aa	1931.6±59.2Ba	1662.2±256.7ABa
UNIJ				
0%	1169.5±187.1Aa	1181.5±19.7Aa	1179.3 ± 13.1Aa	2423.2±207.2Bb
50%	1212.2±54.4Aa	1294.5±68.4Ab	1296.4 ± 68.5Ab	2033.4±228.8Bab
100%	1396.1±123.4Aa	1469.7±39.1Ac	1471.7 ± 35.2Ac	1973.2±155.9Ba
HAB				
0%	641.6±40.1Aa	597.9 ± 97.6Aa	607.7±58.3Aa	
50%	760.9±66.5Aa	719.4 ± 110.5Aa	636.2±69.1Aa	
100%	540.9±6Aa	657.0±208.3Aa	596.8±134.6Aa	

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 water supply according to Tukey's HSD post hoc test.

During the 2019 season (Table 8), water supply had no influence on vitamin C content in HET in the first harvest. However, in the second harvest, vitamin C significantly ($p=0.038$) decreased in 100% and 50% when compared to 0% even though between 50% and 0%, the content did not change. Also, in the third harvest, vitamin C was significantly ($p\leq0.001$) lower in 100% when compared to 50% and 0%. Vitamin C content in the fourth harvest significantly ($p=0.007$) decreased in 50% when compared to 0% even though between 100% and 0%, the content did not differ.

In UNIK, vitamin C significantly ($p=0.003$) decreased at 100% and 50% in the first harvest when compared to 0%. Water supply had no effect on vitamin C content in the second, third and fourth harvests.

In UNIJ, vitamin C significantly ($p=0.033$) increased at 50% and lowest in 100% in the first harvest. In the second harvest, vitamin C significantly ($p=0.005$) decreased at 100% and 50% when compared to 0%. Also, in the third harvest, vitamin C significantly ($p=0.002$) decreased at 100% when compared to 0%. Water supply had no effect on vitamin C content in the fourth harvest.

In HAB, vitamin C was significantly ($p=0.001$) lower in 100% and 50% when compared to 0% in the first harvest. Also, in the second harvest, vitamin C significantly ($p=0.011$) decreased at 50% when compared to 0% even though between 100% and 0%, the content did not differ. In the third harvest, vitamin C significantly ($p=0.012$) decreased at 100% and 50% when compared to 0%.

Table 8: Effect of harvesting periods and water supply treatments on Vitamin C content in 2019 cultivation seasons. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

	Vitamin C 2019			
Water supply treatments/ 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 water supply according to Tukey's HSD post hoc test.

Vitamin C, according to the literature, can be found in fully matured peppers (Howard et al., 2000) and contribute essentially to human nutrition and health (Navarro et al., 2006). Based on the findings, water supply treatments significantly influenced vitamin C content in the peppers cultivated. Under non-irrigated conditions, a high amount of vitamin C was found in HET (4161.0±439.2 µg/g) and decreased as irrigation increased. Deficit irrigated peppers with a lower amount of vitamin C corresponds with a previous study by Ahmed et al. (2014). In relationship to higher amounts of vitamin C in non-irrigated peppers, the use of little or no irrigation treatment can improve the sustainability of the water efficiency programme (Dorji et al., 2005). Aside from HET, higher amounts of vitamin C were present in UNIK (3486.5±515.4 µg/g) and HAB (3223.7±118.6 µg/g), which supports the assertion that vitamin C content in Capsicums is mainly influenced by cultivars (Howard et al., 2000) and growing methods (Pérez-López et al., 2007).

A lower amount of vitamin C was found in HAB (596.8±134.6 µg/g), which in the first year (2018) had yellow-like colour attributes when compared to the second year (2019) HAB (3223.7±118.6 µg/g), which had red-like colour attributes. Higher amounts of vitamin C were evident in red 'Fire flame' hybrid peppers compared to yellow coloured in a previous study by Nagy et al. (2015). Literature indicated that matured peppers contain high amounts of vitamin C but declined in over-

ripened peppers (Gnayfeed et al., 2001) and oxidises very fast when exposed to high to extreme temperatures (Davies et al., 1991).

4.3.3 Tocopherols

The various tocopherol compounds were found in the cultivars for the 2018 season (Table 9). The major compounds, γ-tocopherol, α-tocopherol, and β-tocopherol, had a significant effect ($p<0.05$) on all cultivars (Appendix 3).

Generally, in HET, harvesting periods did not have a significant influence on tocopherols at all levels. Water supply treatments did not influence γ-toc and γ-toc esters. A lower concentration of β-toc and β-toc esters were found in 100% when compared to 0%. The concentration of α-toc quinone was significantly higher in non-irrigated peppers at all harvesting levels. Between 50% and 100%, α-toc QH2 was significantly ($p\leq0.001$) different from each other. The concentration of α-toc and α-toc esters were significantly lower in 100% in the second, third, and fourth harvests.

Water supply treatments did not significantly affect the concentration of γ-toc in UNIK at all harvesting stages. A similar trend was observed in β-toc and β-toc esters in the first and third harvests. A higher concentration of α-toc quinone was found in 0% in the first, fourth, and subsequently in the second harvest (50%). All other minor compounds were found in minimal quantities. However, their concentrations were lower under 100%.

In UNIJ, water supply treatments generally did not significantly influence tocopherol concentration in 50% when compared to 0%. However, higher concentrations of α-toc QH2 were found in 0% but were significantly lower ($p=0.003$) when compared to 100%. Between the harvesting periods, the concentration of tocopherols was found to be significantly lower in the third and fourth harvests when compared to the second and first harvests.

In HAB, higher concentration of α-toc was found in 50% and 100% in the second and third harvests. Water supply treatments had no effect on the concentration of tocopherols. However, minimal amounts were found in 100% but were not significantly different from 0% and 50%.

Table 9: Effect of harvesting periods and water supply treatments on tocopherol compounds in the 2018 season. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Tocopherol group	**Water supply treatments/ Cultivar**	**1st harvest**	**2nd harvest**	**3rd harvest**	**4th harvest**
γ-toc					
	HET				
	0%	0.1±0.1Aa	0.1±0.22Aa	0.1±0.07Aa	0.1±0.04Aa
	50%	0.1±0.01Aab	0.1±0.04Aa	0.1±0.06Aa	0.1±0.03Aa
	100%	0.1±0.03Cb	0.1±0.02Ba	0.0±0.03Aa	0.1±.0.01Ba
	UNIK				
	0%	0.1±0.3Ba	0.1±0.01ABa	0.0±0.05Aa	0.1±0.02Aa
	50%	0.2±0.01Cab	0.1±0.01Ba	0.0±0.01Aa	0.1±0.02Ba
	100%	0.2±0.02Cb	0.1±0.02Ba	0.0±0.04Aa	0.1±0.01ABa
	UNIJ				
	0%	0.1±0.01Ba	0.1±0.02Ba	0.0±.0.03Aa	0.1±0.03Ba
	50%	0.1±0.04Bab	0.1±0.01Aa	0.0±0.04Aab	0.1±0.03ABa
	100%	0.2±0.00Bb	0.1±0.01Aa	0.1±0.02Ab	0.1±0.03Aa
	HAB				
	0%	0.1±0.03Ba	0.0±0.05ABa	0.1±0.06Ba	
	50%	0.1±0.02Ba	0.0±0.04Aa	0.1±0.01Ba	
	100%	0.1±0.02Ca	0.0±0.01Aa	0.1±0.01Ba	
β-toc					
	HET				
	0%	0.9±0.12Ba	0.8±0.24ABa	0.5±0.16Aa	1.0±0.18Ba
	50%	1.0±0.11Aa	0.6±0.12Aa	0.4±0.09Aa	2.0±2.32Aa
	100%	1.5±0.29Bb	0.5±0.07Aa	0.5±0.23Aa	0.8±0.31Aa
	UNIK				
	0%	1.8±0.44Ba	0.9±0.15Aa	0.7±0.08Aa	2.2±0.55Ba
	50%	1.7±0.18Ba	1.3±0.34ABa	0.7±0.10Aa	1.2±0.82ABa
	100%	2.0±0.66Ba	1.1±0.21ABa	0.7±0.41Aa	1.6±0.42ABa
	UNIJ				
	0%	0.7±0.27Ba	0.2±0.10Aa	0.1±0.03Aa	0.2±0.10Aa
	50%	0.6±0.11Ba	0.2±0.03Aa	0.1±0.04Aa	0.2±0.11Aa
	100%	1.4±0.84Ba	0.4±0.13Aa	0.1±0.06Aa	0.2±0.08Aa
	HAB				
	0%	0.3±0.11Aa	0.1±0.02Aa	1.0±0.28Ba	
	50%	0.4±0.15Ba	0.1±0.02Aa	0.6±0.13Ba	
	100%	0.3±0.10ABa	0.2±0.40ABa	0.6±0.20Ba	
α-toc QH2					
	HET				
	0%	50.0±11.1Ba	34.3±3.36Ab	20.8±6.43Aa	29.7±6.07Aa
	50%	43.1±9.09Ca	29.0±5.53Bab	15.6±2.07Aa	29.5±5.34Ba
	100%	59.2±10.12Ba	25.2±4.11Aa	18.5±5.11Aa	29.8±5.22Aa
	UNIK				
	0%	64.6±9.11Ca	33.7±3.22Ba	20.2±4.28Aa	38.0±2.92Ba
	50%	54.2±12.31Ca	35.3±3.84Ba	19.8±1.60Aa	33.8±1.79Ba
	100%	60.0±17.0Ba	32.5±3.79Aa	19.8±11.17Aa	35.5±1.73ABa
	UNIJ				
	0%	21.7±9.50Ba	10.0±4.97Aa	3.5±1.24Aa	6.8±1.42Aa
	50%	13.3±2.85Ca	7.8±1.00Ba	2.9±1.51Aa	5.7±2.63ABa
	100%	33.7±19.79Ba	13.0±4.28ABa	4.1±2.56Aa	4.1±2.56Aa
	HAB				
	0%	11.9±3.04Ba	2.8±1.16Aa	21.9±6.58Ca	

	50%	15.6±5.91Ba	4.8±0.74Aa	20.9±4.76Ba	
	100%	12.0±3.46Aa	9.4±11.42ABa	19.1±3.92Ba	
α-toc					
	HET	76.7±6.17Ba	43.0±1.30Ab	40.0±6.27Ab	33.4±7.51Aa
	0%	79.7±1.95Ba	43.0±0.73Ab	32.0±3.85Aab	37.0±12.54Aa
	50%	81.4±4.60Ca	38.5±3.51Ba	26.5±7.02Aa	38.7±2.67Ba
	100%				
	UNIK				
	0%	77.1±6.31Ba	42.3±2.55Aa	37.1±7.64Ab	41.9±4.26Aa
	50%	73.4±10.36Ca	41.1±4.59Ba	27.1±2.98Aab	36.6±4.38ABa
	100%	76.3±2.71Ca	38.4±1.00Ba	25.2±3.14Aa	41.5±3.62Ba
	UNIJ				
	0%	50.2±20.36Ba	41.26±1.55Abb	22.7±5.48Aa	23.1±12.04Aa
	50%	43.6±18.43Aa	32.8±5.69Aa	21.7±5.21Aa	28.1±11.45Aa
	100%	54.8±14.40Ba	34.8±3.25Aab	17.5±7.43Aa	30.3±9.00Aa
	HAB				
	0%	23.6±7.09Ba	7.10±4.83Aa	25.7±3.15Ca	
	50%	21.9±7.20Ba	10.4±1.15Aa	30.8±1.13Ba	
	100%	14.2±8.30Ba	10.1±9.73ABa	28.3±3.40Ba	
α-toc ester					
	HET				
	0%	9.8±2.30Ba	6.5±0.61ABb	3.6±0.58Aa	7.5±1.90Ba
	50%	10.2±2.41Ba	6.4±0.55ABb	3.5±0.43Aa	9.0±2.70Ba
	100%	10.1±1.60Ba	5.0±0.76Aa	3.0±0.50Aa	9.3±0.81Ba
	UNIK				
	0%	7.8±0.74Ba	4.6±0.97Aa	3.5±0.57Aa	8.6±1.60Ba
	50%	7.2±2.18Ba	4.9±1.60ABa	3.14±0.50Aa	6.6±1.64Ba
	100%	8.6±1.10Ba	3.7±0.43Aa	2.7±0.74Aa	9.0±1.41Ba
	UNIJ				
	0%	3.0±1.19ABa	3.3±0.70ABa	1.6±0.38Aa	3.9±1.06Ba
	50%	3.0±0.45BCa	2.3±0.55ABa	1.5±0.35Aa	4.3±1.00Ca
	100%	3.6±0.55Ba	2.6±0.70ABa	1.3±0.70Aa	5.2±0.70Ca
	HAB				
	0%	1.3±0.3ABa	0.3±0.12Aa	2.2±1.43Ba	
	50%	1.3±0.40Ba	0.4±0.02Aa	2.0±0.17Ca	
	100%	1.2±0.08ABa	0.8±1.24ABa	1.8±0.70Ba	

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; ND: not detected; γ-toc: γ-tocopherol; β-toc: β-tocopherol; α-toc QH2: α-toc hydro quinone: α-tocopherol quinone; α-toc: α-tocopherol; α-toc ester: α-tocopherol ester.

During the 2019 growing period (Table 10), HET had a lower concentration of γ-toc in the first and fourth harvests under all water supply treatments. However, in the second and third harvest, γ-toc were absent. β-toc concentration was found to be significantly ($p=0.007$) higher in the third harvest in 50% and 100% when compared to 0%. A higher concentration of α-toc was found in 0% and lowered in 100%. However, between 100% and 50%, water supply treatments did not significantly affect α-toc concentration. β-toc ester was absent in the first and second harvests but minimal amounts in the third and fourth harvests.

UNIK had a lower concentration of γ-toc and γ-toc ester at all harvesting stages. Besides, the water supply treatments did not influence their concentration. Similarly, β-toc recorded lower concentration, and α-toc ester was absent. The concentration of α-toc QH2 and α-toc were found to be higher in 0% ($p \leq 0.001$) and significantly lower in 100% ($p=0.001$).

UNIJ recorded a significantly lower concentration of γ-toc, β-toc, and α-toc ester. The concentration of α-toc hydroquinone and α-toc were present at all harvesting stages and significantly higher in 0% when compared to 100%. However, at 50%, the concentrations of α-toc quinone and α-toc were not significantly different when compared to 0%.

HAB, on the other hand, recorded a lower concentration of tocopherols at all harvesting stages. A general decline in tocopherol concentration was observed in 100%.

Table 10: Effect of harvesting periods and water supply treatments on tocopherol compounds during 2019. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Tocopherol group	**Water supply treatments/ Cultivar**	**1st harvest**	**2nd harvest**	**3rd harvest**	**4th harvest**
γ-toc					
	HET				
	0%	0.0±0.05Aa	0.1±0.20Aa	0.1±0.12Aa	0.1±0.03Aa
	50%	0.1±0.04Aa	ND	ND	0.2±0.14Aa
	100%	0.0±0.01Aa	0.1±0.16Aa	ND	0.1±0.09Aa
	UNIK				
	0%	0.1±0.04Aa	0.0±0.02Aa	0.0±0.01Aa	0.1±0.16Aa
	50%	0.0±0.07Aa	0.0±0.0Aa	0.1±0.02Aa	0.0±0.02Aa
	100%	ND	0.0±0.01Aa	0.01±0.01Aa	0.1±0.08Aa
	UNIJ				
	0%	0.1±0.04Aa	0.1±0.01Aa	0.1±0.01Aa	0.0±0.01Aa
	50%	0.0±0.02Aa	0.0±0.00Aa	0.0±0.01Aa	0.0±0.01Aa
	100%	tr	tr	0.1±0.02Aa	tr
	HAB				
	0%	0.1±0.01Aa	0.1±0.01Aa	0.1±0.02Aa	
	50%	0.1±0.03Bab	0.1±0.03Ba	0.0±0.01Aa	
	100%	0.1±0.03Bb	0.1±0.03Ba	0.1±0.02Aa	
β-toc					
	HET				
	0%	0.5±0.30Aa	0.6±0.11Aa	0.6±0.14Aa	1.0±0.14Aa
	50%	0.3±0.10Aa	0.5±0.08ABa	0.7±0.10Bab	0.7±0.22Ba
	100%	0.6±0.12Aa	0.7±0.10ABa	1.0±0.10BCb	1.0±0.15Ca
	UNIK				
	0%	0.6±0.40Aa	0.7±0.34Aa	1.1±0.32ABa	1.5±0.45Ba
	50%	1.0±0.13Aa	1.1±0.45ABab	1.4±0.26ABa	1.5±0.17Ba
	100%	1.0±0.13Aa	1.5±0.11ABb	1.8±0.51Ba	1.7±0.31Ba
	UNIJ				
	0%	0.1±0.08Aa	0.2±0.01ABa	0.3±0.08Bab	0.2±0.08ABa
	50%	0.1±0.04Aa	0.2±0.03ABa	0.4±0.04Cb	0.3±0.06Ba
	100%	0.5±0.06Aa	0.3±0.06Aa	0.2±0.06Aa	0.2±0.07Aa

	HAB				
	0%	0.2±0.08BCa	0.3±0.05Ca	0.1±0.10ABa	
	50%	0.2±0.06ABa	0.4±0.22Ba	0.5±0.20Bb	
	100%	0.2±0.02Aa	0.6±0.20Ba	0.5±0.05Bb	
α-toc QH2					
	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±12.74Aa
	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	
α-toc 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	1.8±0.62ABa	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 water supply according to Tukey's HSD post hoc test.

For their lipid-soluble characteristics, tocopherols (vitamin E) are very useful in free radical oxidation (DellaPenna, 2005; Shintani & DellaPenna, 1998). It was observed in the first year that γ-tocopherol, α-tocopherol, and β-tocopherol had a significant effect ($p<0.05$) on all cultivars (HET, UNIK, UNIJ, and HAB) under 0% (Table 9). However, lower concentration of γ-toc and β-toc was observed in all cultivars irrespective of the water supply treatments, but UNIK (2.2±0.5 µg/g) and HET (2.0±2.32 µg/g) recorded concentration of β-toc under 0% and 50%. Also, α-toc concentration was significantly lower in 100%. The concentration of α-toc under 0% was found to be higher in HET (between 76.6±6.17 µg/g – 81.4±4.60 µg/g) and lower in HAB (between 7.10±4.8 µg/g – 10.4±1.15 µg/g) during the first growing season (Table 9). The red-like colour attribute of HET and yellow-like colour attribute of HAB corresponded with a previous study by Koch et al. (2002) when higher levels of tocopherols in red pepper pericarp were reported.

By the second growing season, a decrease in tocopherol concentration was observed in all cultivars (HET, UNIK, UNIJ, and HAB) (Table 10). A lower concentration of γ-toc was evident in all cultivars irrespective of the water supply treatments. The concentration of γ-toc is known to be higher in paprika seeds (Márkus et al., 1999). However, this was not the case in this study. The concentration of α-toc was found to be higher in 0% in HET (between 58.1±7.1 µg/g – 69.0±1.7 µg/g) and lower in HAB (between 1.7±0.78 µg/g – 0.1±0.03 µg/g). β-toc was absent or in minimal amounts in all cultivars (Table 9). Other tocopherol concentration was found to be low, especially under 100% optimum water supply.

From the study in both years, categorising tocopherol compounds, mostly α-tocopherol represents a notable dominant composition mainly found in plant tissues and during their fruit development stage (DellaPenna, 2005). In a study by Navarro et al. (2006), ripened peppers contain high antioxidant composition, which corroborates our findings that all cultivars had a higher concentration of tocopherols in the first three harvests. However, lower concentration or absence of tocopherols by the fourth harvest may be as a result that, though fruits were ripened, their maturity age was not achieved.

The decline in tocopherols by the second year, especially in HAB, may be a result of lipid oxidation which is a significant cause for fruit quality deterioration, which decreases the nutritive value of food (Alamed et al., 2009). A cut-through in HAB prior to analyses showed that seeds quite deteriorated (Márkus et al., 1999). It was also observed in this study that 50% had minimal influence on tocopherol concentration. This indicates that a 50% deficit may not be relevant for tocopherols (vitamin E) since it did not increase or decrease tocopherol concentration.

4.3.4 Carotenoids

The individual peaks were identified on the chromatogram (Appendix 5). The concentration of carotenoid compounds for the year 2018 was assessed (Table 11).

Water supply had no effect on free caps concentration in the first and fourth harvests. In the second harvest, a significantly ($p=0.009$) lower concentration was found in 100% when compared to 50% and 100%. Also, in the third harvest, a significantly ($p=0.033$) lower concentration was found in 100% but was not different from 50%. Water supply had no influence on free caps in UNIK in the first, second and fourth harvests. However, in the third harvest, a significantly ($p=0.025$) lower concentration was found in 100% when compared to 0%. Nonetheless, free caps concentration in 50% was not different from 100% and 0%. In UNIJ, water supply had no effect on free caps in the first and third harvests. Concentration was significantly ($p=0.007$) lower in 100% and 50% when compared to 0% in the second harvest. A significantly ($p=0.027$) higher concentration in 100% in the fourth harvest. Free caps concentration in HAB was significantly ($p\leq0.001$) higher in 50% and 100% in the first harvest. Also, in the second harvest, water supply significantly ($p=0.026$) influenced free caps concentration. However, in the third harvest, water supply had no influence on concentration.

Water supply had no effect on free zeax concentration in HET in all harvest periods. In UNIK, water supply did not influence free zeax concentration in the first and second harvests. However, in the third harvest, a significantly ($p\leq0.001$) lower concentration was found in 100% and 50% when compared to 0%. Also, in the fourth harvest, a significantly ($p=0.037$) lower concentration was found in the 50% deficit. Water supply had no free zeax concentration in UNIJ in all harvest periods. In HAB, free zeax was significantly ($p=0.009$) lower in 50% in the first harvest, but in the third harvest, water supply had no influence on the concentration.

Caps ME in HET was not influenced by water supply in the first, third and fourth harvests. Nonetheless, caps ME was significantly ($p=0.004$) lower in 100% when compared to 50% and 0%. In UNIK, water supply had no influence on caps ME at all harvest periods. Water supply had no effect on caps ME in UNIJ in the first and third harvests. However, in the second harvest, caps ME was significantly ($p\leq0.001$) lower in 100% and 50% when compared to 0%. Also, in the fourth harvest, concentration was significantly ($p=0.036$) lower in 100% optimum water when compared to 50% deficit. In HAB, water supply had no effect on caps ME in the first and third harvests.

Nevertheless, a significantly (p=0.050) lower concentration was found in 100% when compared to 0% in the second harvest.

Water supply had no influence on zeax ME concentration in HET in all harvest periods. Also, in the UNIK, water supply had no effect on zeax ME in all harvest periods. In UNIJ, water supply had no effect on zeax ME in the first and third harvests. However, in the second harvest, a significantly (p=0.011) lower zeax ME was found in 50% and 100% when compared to 0%. Also, water supply significantly (p=0.010) influenced concentration in the fourth harvest. In HAB, water supply had a significant (p=0.015) influence on zeax ME concentration in the second harvest.

Beta-carotene in HET was not influenced by water supply in all harvests. Similarly, water supply had no effect on β-carotene in UNIK in all harvests. In UNIJ, water supply did not influence β-carotene in the first and third harvests. However, in the second harvest, a significantly (p=0.001) lower β-carotene was detected in 100% and 50% when compared to 0%. Also, water supply significantly (p=0.005) influenced β-carotene in the fourth harvest.

In HET, water supply had no effect on caps DE concentration in all harvests. Also, in UNIK, water supply did not influence caps DE in all harvest periods. In UNIJ, a significantly (p=0.021) higher caps DE was found in the 50% deficit in the first harvest. In the second harvest caps DE was significantly (p=0.002) lower in 50% and 100% when compared to 0%. A significantly (p=0.015) higher concentration was recorded in 100% in the fourth harvest. Water supply did not influence caps DE concentration in HAB in the first and third harvests. However, in the third harvest, a significantly (p=0.004) lower caps DE in 100% when compared to 0%.

Zeax DE in HET was significantly (p=0.05) lower in 100% in the first harvest. Also, in the second harvest, a significantly ($p \leq 0.001$) higher zeax DE was recorded in 100% when compared to 50% and 0%. However, in the third harvest, water supply had no effect on zeax DE concentration. A significantly (p=0.002) higher zeax DE was found in 50% when compared to 0% and 100%. In UNIK, a significantly (p=0.050) higher concentration was recorded in 100% when compared to 0% in the first harvest. Nevertheless, water supply had no influence on the other harvest periods. Water supply had no effect on zeax DE in UNIJ in the first and second harvests. However, in the fourth harvest, a significantly (p=0.014) higher concentration was detected in 100% optimum water. In HAB, water supply had a significantly ($p \leq 0.001$) higher zeax DE was recorded in 100% optimum water when compared to 0% and 50% deficit.

Table 11: Effect of harvesting periods and water supply treatments on carotenoid concentration in the 2018 season. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Carotenoid	**Water supply treatments/ Cultivar**	**1st harvest**	**2nd harvest**	**3rd harvest**	**4th harvest**
Free caps					
	HET				
	0%	17.0±5.0Aa	36.9±5.4Bb	46.8±8.7Bb	14.9±9.4Aa
	50%	20.8±6.6Aa	35.4±3.0Bb	34.3±5.4Bab	20.1±5.5Aa
	100%	21.6±4.4Aa	26.5±2.7Aa	28.5±9.9Aa	16.6±1.8Aa
	UNIK				
	0%	29.1±7.9Aa	42.8±7.6Aa	64.6±11.2Bb	36.4±7.5Aa
	50%	26.4±7.2Aa	50.6±19.5Ba	55.9±8.0Bab	24.9±5.7Aa
	100%	26.0±2.3Aa	35.3±9.0ABa	42.9±7.8Ba	31.5±4.7ABa
	UNIJ				
	0%	14.7±6.1Aa	48.2±11.5Cb	35.7±4.1BCa	24.0±6.2ABab
	50%	20.1±5.3Aa	24.7±10.0Aa	31.7±9.9Aa	18.0±1.8Aa
	100%	13.9±9.4Aa	23.0±5.4ABa	27.5±2.9Ba	30.2±6.2Bb
	HAB				
	0%	1.9±0.2Bb	0.4±0.1Aab	0.4±0.1Aa	
	50%	0.6±0.1Ba	0.5±0.2ABb	0.4±0.0Aa	
	100%	0.6±0.1Ba	0.2±0.01Aa	0.3±0.1Aa	
Free zeax					
	HET				
	0%	2.3±1.4Aa	4.1±1.5Aa	0.8±0.1Aa	4.2±2.8Aa
	50%	2.4±0.4Ba	2.3±0.3Ba	0.6±0.2Aa	4.9±1.1Ca
	100%	2.1±0.6Aa	2.5±0.8Aa	3.5±3.6Aa	5.2±1.8Aa
	UNIK				
	0%	5.2±1.4Aa	5.8±1.4Aa	6.1±3.8Bb	7.7±1.6Ab
	50%	5.8±1.8Ba	5.4±2.3Ba	0.5±0.2Aa	4.7±1.6Ba
	100%	4.3±2.6Ba	3.8±1.4Ba	0.3±0.1Aa	5.8±0.9Bab
	UNIJ				
	0%	4.9±2.6Ba	7.7±2.9BCa	0.3±0.1Aa	11.5±1.6Ca
	50%	5.6±1.4Ba	5.9±1.7Ba	0.2±0.1Aa	11.1±2.2Ca
	100%	5.1±2.2Ba	6.6±0.9Ba	0.2±0.1Aa	13.9±2.6Ca
	HAB				
	0%	0.4±0.2Bb	0.1±0.1Aa	ND	
	50%	0.1±0.1Aab	0.1±0.0Aa	ND	
	100%	ND	tr	ND	
Caps ME					
	HET				
	0%	37.2±5.7Aa	54.5±6.5Aa	87.7±27.8Ab	32.5±8.1Aa
	50%	30.3±10.6Aa	53.7±5.5Aab	70.58±8.3Aa	35.9±13.6Aa
	100%	29.6±6.0Aa	37.6±5.2Ba	59.49±14.6Ab	28.2±6.9Aa
	UNIK				
	0%	25.0±7.9Aa	40.2±8.7Aa	77.9±17.3Ba	31.1±9.8Aa
	50%	23.3±10.5Aa	46.4±18.2ABa	58.3±13.6Ba	21.1±8.7Aa
	100%	16.7±6.4Aa	36.1±7.1Ba	55.3±9.6ABa	26.3±7.1Ca
	UNIJ				
	0%	12.9±8.8Aa	54.2±7.1Bb	55.6±14.8Ba	18.3±9.8Aab
	50%	20.7±4.2ABa	27.9±6.7Ba	48.6±12.0Ca	7.0±2.7Aa
	100%	9.8±6.9Aa	27.4±5.2ABa	42.5±10.7Ba	31.5±16.2ABb
	HAB				
	0%	1.2±0.9Ba	0.7±0.5ABab	0.9±0.2ABa	

	50%	0.7±0.2Aa	1.0±0.1Ab	0.9±0.2Aa	
	100%	0.5±0.1Aa	0.4±0.2Aa	0.5±0.2Aa	
Zeax ME					
	HET				
	0%	5.2±1.0Aa	7.6±0.6Aa	23.6±10.9Ba	15.8±4.5ABa
	50%	6.1±2.7Aa	8.0±0.7ABa	13.2±4.2Ba	13.6±3.3Ba
	100%	7.5±3.2Aa	8.6±2.7Aa	15.9±9.6Aa	14.8±7.4Aa
	UNIK				
	0%	5.9±1.2Aa	11.2±2.1Aa	28.2±9.9Ba	14.7±3.6Aa
	50%	4.4±2.3Aa	15.0±13.4Aa	17.0±2.8Aa	10.7±2.0Aa
	100%	2.7±1.9Aa	8.1±1.2Aa	17.4±3.7Ba	16.0±3.5Ba
	UNIJ				
	0%	6.0±4.2Aa	23.0±3.4Bb	7.6±1.4Aa	12.9±5.9Aab
	50%	7.7±1.4Aa	15.7±2.5Ba	6.3±1.2Aa	7.2±2.9Aa
	100%	6.1±5.0Aa	17.0±2.2Ba	5.7±1.1Aa	19.9±4.0Bb
	HAB				
	0%	ND	0.04±0.03a	ND	
	50%	ND	ND	ND	
	100%	ND	ND	ND	
β-carotene					
	HET				
	0%	17.0±4.1Aa	37.7±7.3ABa	74.4±20.8Ca	63.7±20.6BCa
	50%	16.6±3.2Aa	37.1±3.7Ba	63.3±7.4Ca	68.1±16.8Ca
	100%	15.8±2.8Aa	31.2±9.8Aa	58.6±13.6Ba	58.0±11.6Ba
	UNIK				
	0%	17.2±5.0Aa	35.9±5.4Aa	84.9±23.9Ba	64.0±9.5Ba
	50%	17.0±7.1Aa	36.9±16.6ABa	57.3±8.8Ba	43.5±17.4ABa
	100%	9.6±6.3Aa	33.0±5.1Ba	60.3±8.0Ca	51.7±7.4Ca
	UNIJ				
	0%	10.3±8.0Aa	63.2±2.8Cb	58.4±13.8Ca	33.7±11.8Bab
	50%	16.2±3.4Aa	39.8±8.0Ba	60.1±15.9Ca	19.5±4.5Aa
	100%	9.6±6.6Aa	37.3±7.6Ba	55.0±16.6Ba	55.4±14.7Bb
	HAB				
	0%	ND	0.01±0.0Aa	ND	
	50%	ND	0.1±0.0Aa	ND	
	100%	0.2±0.1Aa	0.04±0Aa	tr	
Caps DE					
	HET				
	0%	152.2±27.2Aa	202.9±26.4ABa	298.2±106.2Ba	192.8±39.6ABa
	50%	158.1±27.8Aa	225.2±22.5Ba	301.0±10.1Ca	187.5±29.3ABa
	100%	156.1±19.2Aa	178.1±36.3ABa	264.9±55.6Ba	153.0±57.1Aa
	UNIK				
	0%	71.6±13.7Ba	128.2±14.9Aa	ND	123.6±25.1Ca
	50%	66.0±29.8ABa	136.8±56.8Ba	4.9±9.7Aa	83.5±33.8Ba
	100%	31.2±20.3Aa	120.4±33.8Ba	3.2±6.5Aa	109.7±27.5Ba
	UNIJ				
	0%	41.8±23.1Bab	177.8±16.9Cb	ND	63.8±27.6Bab
	50%	70.3±16.6Cb	114.3±22.2Da	ND	37.2±12.8Ba
	100%	26.3±13.1Aa	115.0±19.9Ba	ND	116.1±43.4Bb
	HAB				
	0%	3.6±2.1ABa	2.2±0.5ABb	7.0±5.1Ba	
	50%	4.9±2.3Ba	1.4±0.5Aab	9.0±1.5Ca	
	100%	5.7±1.6Ba	0.8±0.3Aa	7.1±3.4Ba	
Zeax DE					
	HET				
	0%	8.5±2.1ABb	9.7±5.6ABa	18.3±10.6Ba	1.2±0.5Aa
	50%	6.7±0.5Aab	9.0±1.7Aa	11.9±2.1ABa	17.8±7.8Bb

	100%	4.9±2.2Ba	7.1±3.3Aa	9.2±6.8Aa	3.8±2.6Aa
	UNIK				
	0%	13±0.9Ba	7.7±0.7Ba	ND	7.0±5.7Ba
	50%	14.9±2.6Bab	6.3±4.8Aa	ND	5.1±4.7Aa
	100%	17.2±2.3Cb	5.1±1.9ABa	ND	6.8±3.9Ba
	UNIJ				
	0%	6.9±0.5ABa	31.3±8.0Ca	ND	11.2±5.6Bab
	50%	8.5±0.9Aa	22.6±4.5Ca	ND	7.3±6.1ABa
	100%	6.5±5.9Aa	24.6±5.9Ba	ND	21.9±5.2Bb
	HAB				
	0%	2±0.3Aa	0.1±0.0Aa	ND	
	50%	1.1±1.2Aa	ND	ND	
	100%	1.3±0.8Ba	ND	0.2±0.0Aa	

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; ND: not detected; tr: traces; Free caps: Free capsanthin; Free zeax: Free zeaxanthin; Caps ME: Capsanthin mono ester; Zeax ME: Zeaxanthin mono ester; β-carotene: Beta carotene; Caps DE: Capsanthin di-ester; Zeax DE: Zeaxanthin di-ester.

Total carotenoid concentration represents the sum of individual peaks identified on the chromatogram. A lower concentration of carotenoid compounds was found in the first harvest when compared to the second, third, and fourth harvests. Between the cultivars, higher concentrations of total carotenoids were found in HET and UNIJ when compared to UNIK and HAB. The concentration of HAB was lowest when compared to the other pepper cultivars (fig. 13).

In the first harvest, no effect was found in HET and in UNIK, even though a slight decrease in water supply was recorded. Higher concentrations of carotenoids were recorded in UNIJ deficit irrigated peppers and significantly ($p=0.018$) lower in the optimum water supply. Similar to the first harvest, HET and UNIK had no significant differences in the water supply. However, in UNIJ, the carotenoid concentration decreased significantly ($p\leq0.001$) as water supply increased even though between 50% and 100%, no significant differences were found. In HAB, carotenoid concentration increased significantly ($p=0.025$) at 100% when compared to 0%.

As water supply increased, total carotenoid concentration slightly decreased in cultivars in the third harvest. HET had higher amounts of carotenoids, and HAB had the lowest concentration. Water supply treatments had no influence on HET and UNIK in the fourth harvest. However, in UNIJ, carotenoid concentrations were higher in 100% and significant ($p=0.031$) lower in 50%. On the other hand, HAB had a significantly ($p=0.045$) lower concentration in 100% when compared to 0%. (fig 13).

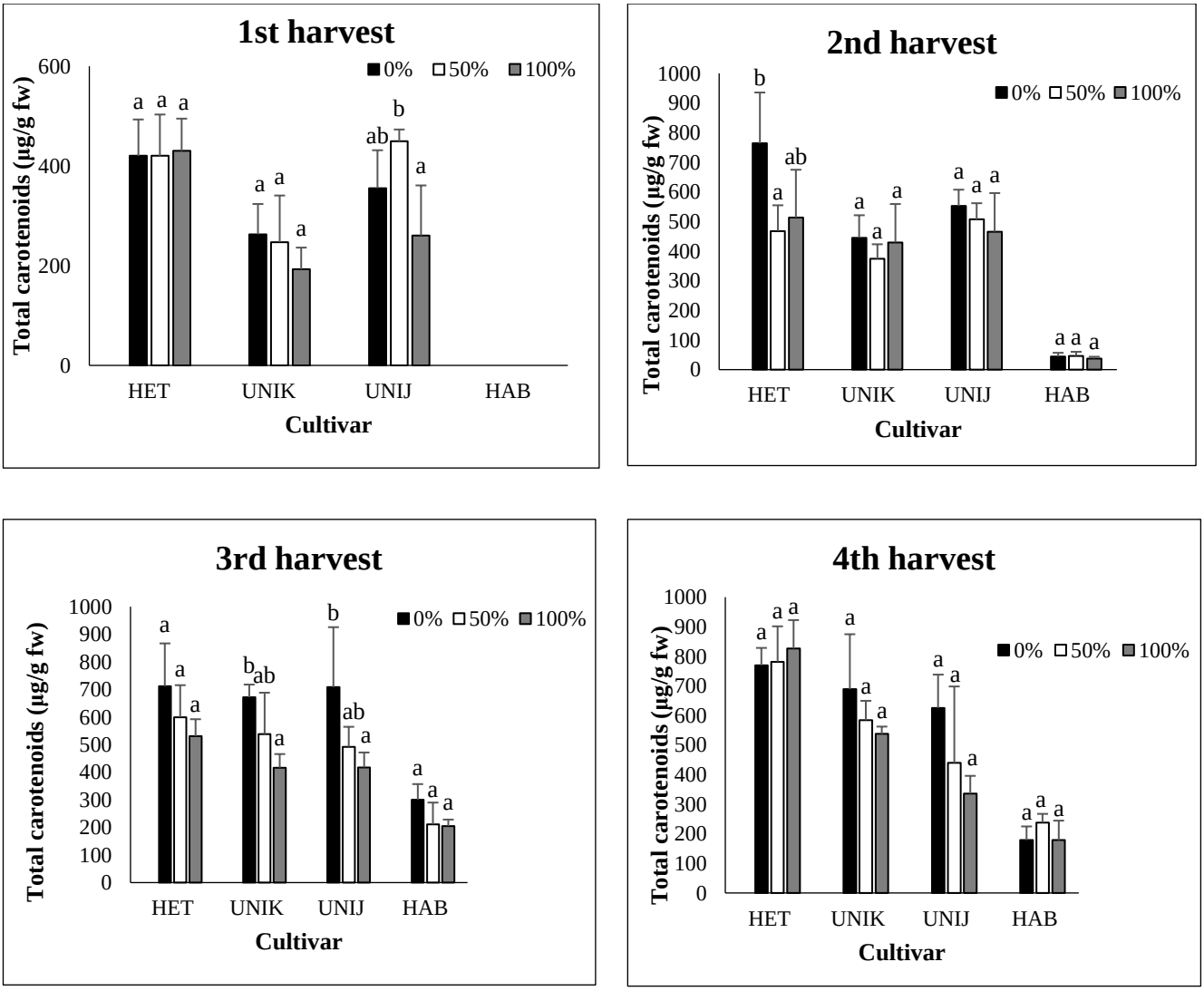


Figure 13. Mean concentration of total carotenoids (µg/g fw) present in the chilli pepper cultivars at the various harvesting stages in the 2018 growing season.

Carotenoid compounds in the 2019 season (Table 12) showed that water supply had no effect on free caps in HET in the first and third harvest periods. However, in the second harvest, free caps concentration was significantly (p=0.007) lower in 50% and 100% when compared to 0%. Also, free caps were significantly (p=0.004) lower in 100% when compared to 0% in the fourth harvest even though at 50%, concentration did not differ from that of 100% and 0%. Water supply had no effect on free caps in UNIK in the first, second and fourth harvest periods. However, in the third harvest, concentration was significantly (p=0.005) lower in 100% and 50%. In UNIJ, water supply had no influence on free caps in all harvest periods. Also, in HAB, water supply had no effect on concentration in all harvests.

Water supply did not influence free zeax concentration in HET in the first and second harvests. However, free zeax significantly ($p=0.036$) decreased at 100% when compared to 0% even though concentration at 50% was not different from that of 100% and 0%. Nonetheless, free zeax significantly ($p\leq0.001$) decreased at 50% and 100% in the fourth harvest when compared to 0%. In UNIK, water supply had no effect on free zeax in the first and second harvest periods, but in the third harvest, concentration was significantly ($p=0.043$) lower in 100% when compared to 0%. Also, in the fourth harvest, free zeax was significantly ($p=0.023$) lower in 50% and 100% when compared to 0%. In UNIJ, water supply had no influence on free zeax concentration in all harvests. Minimal or traces of free zeax were detected in HAB in all harvests.

Water supply had no effect on caps ME in HET in the first, third and fourth harvests. However, a significantly ($p=0.021$) lower concentration was found in 50% when compared to 0% even though concentration in 100% was not different from that of 50% and 100%. In UNIK, a significantly ($p=0.024$) lower caps ME was found in 100% when compared to 0% in the first harvest. However, concentration in 50% was not different from that of 100% and 0%. A significantly higher caps ME was recorded in 50% and 100% of the second ($p=0.007$) and fourth ($p\leq0.001$) harvests. Nevertheless, in the third harvest, water supply had no effect on caps ME concentration. IN UNIJ, water supply had no influence on caps ME in all harvests. In HAB, a significantly ($p=0.041$) lower caps ME was recorded in 100% even though concentration was not different from that of 0% in the first harvest. However, in the second and third harvests, water supply had no influence on concentration.

Water supply had no effect on zeax ME in HET, UNIK and HAB in all harvest periods. In UNIJ, a significantly ($p=0.001$) lower zeax ME was detected in 100% and 50% when compared to 0% in the second harvest. However, in the first, third and fourth harvests, water supply had no effect on zeax ME.

In HET, β-carotene was significantly ($p=0.003$) lower in 100% when compared to 50% and 0% in the first harvest. In the second and fourth harvests, water supply had no effect on concentration. However, in the third harvest, β-carotene was significantly ($p=0.030$) lower in 50%. Beta-carotene in UNIK was significantly ($p=0.033$) lower in 100% when compared to 0% in the first harvest. Also, in the fourth harvest, β-carotene was significantly ($p=0.001$) lower in 50% and 100%. However, in the second and third harvests, concentration was not affected by water supply. In UNIJ, water supply had no effect on β-carotene in the first and second harvests. Concentration was significantly lower in 100%, and 50% in the third ($p=0.023$) and fourth ($p=0.002$) harvests

when compared in 0%. In HAB, β-carotene was not affected by water supply in the first and second harvests. However, in the third harvest, concentration was significantly ($p=0.003$) lower in 100% and 50% when compared to 0%.

Caps DE was significantly ($p=0.042$) lower in 100% when compared to 0% in the first harvest. In the second harvest, concentration was significantly ($p=0.029$) lower in 50%. However, in the third and fourth harvests, water supply had no influence on caps DE concentration. In UNIK, caps DE was significantly lower in 100% in the first ($p=0.029$) and third ($p=0.012$) harvests even though concentration in 50% was not different from that of 100% and 0%. Water supply had no effect on concentration in the second and fourth harvests. In UNIJ, a significantly ($p=0.018$) lower caps DE was recorded in 100% in the first harvest. In the second harvest, a significantly ($p\leq0.001$) lower concentration was found in 50%. Also, in the third harvest, caps DE was significantly ($p=0.005$) lower in 100% and 50% when compared to 0%. However, in the fourth harvest, water supply had no influence on caps DE. Caps DE in HAB was significantly ($p=0.006$) higher in 100% and 50% in the first harvest. However, in the second harvest, concentration was significantly ($p=0.007$) lower in 50% and 100%. Nonetheless, water supply had no effect on concentration in the third harvest.

In HET, zeax DE was significantly($p=0.006$) higher in 50% when compared to 0% and 100% in the first harvest. No effect was recorded in the second and fourth harvests. However, in the third harvest, zeax DE was significantly ($p\leq0.001$) higher in 100%. Water supply had no influence on zeax DE concentration in UNIK in the first and second harvests. Concentration was significantly ($p\leq0.001$) lower in 100% and 50% in the third harvest. In the fourth harvest, concentration was significantly ($p=0.021$) higher in 50%. In UNIJ, water supply had no effect on zeax DE in the first and fourth harvests. In the second harvest, concentration was significantly ($p\leq0.001$) lower in 50% and 100%. However, in the third harvest, a significantly ($p=0.002$) higher concentration was found in 100% and 50%. A lower zeax DE concentration in HAB was significant in the second ($p=0.032$); however, minimal amount or traces of zeax DE was significant ($p=0.017$) in the third harvest.

Table 12: Effect of harvesting periods and water supply treatments on carotenoid concentration in the 2019 season. The means are expressed in µg/g fresh base weight ± S.D (n = 4).

Carotenoid	**Water supply treatments/ Cultivar**	**1st harvest**	**2nd harvest**	**3rd harvest**	**4th harvest**
Free caps					
	HET				
	0%	0.6±0.3Aa	1.9±0.4BCb	0.4±0.1Ba	5.4±0.9Cb
	50%	0.8±0.3Aa	0.9±0.3Aa	0.4±0.1Ba	0.4±0.2Cab
	100%	0.9±0.4Aa	1.2±0.4ABa	0.2±0.0ABa	0.4±0.1Ba
	UNIK				
	0%	1.1±0.5Aa	1.1±0.5Aa	3.5±0.5Bb	3.6±2.0Ba
	50%	1.1±0.4Aa	1±0.2Aa	1.9±0.5Aa	3.2±0.9Ba
	100%	0.9±0.2Aa	1.7±0.8Aa	1.9±0.7ABa	2.4±0.5Ba
	UNIJ				
	0%	0.5±0.4Aa	1.3±0.5Aa	2.0±1.3Aa	0.6±1.0Aa
	50%	0.2±0.1Aa	1.2±0.6Aa	1.1±0.8Aa	0.7±0.7Aa
	100%	0.2±0.1Aa	0.8±0.6ABa	1.5±0.9Ba	0.7±0.4ABa
	HAB				
	0%	0.3±0.0Ab	0.1±0.0Aa	0.1±0.0Aa	
	50%	0.1±0.0Aa	0.3±0.1Ba	0.7±0.1Ba	
	100%	ND	0.1±0.0Aa	0.4±0.1Aa	
Free zeax					
	HET				
	0%	12±3.9Ba	15.1±3.5Ba	0.4±0.1Ab	5.4±0.9Ab
	50%	15.8±2.2Ca	8.8±2.0Ba	0.4±0.1Aab	0.4±0.2Aa
	100%	18.9±6.3Ca	9.4±3.9Ba	0.2±0.0Aa	0.4±0.2Aa
	UNIK				
	0%	16.1±9.9Ba	10.6±2.4ABa	0.8±0.1Ab	3.2±1.9Ab
	50%	25.9±6.9Ca	8.7±0.9Ba	0.6±0.2Aab	0.8±0.2Aa
	100%	24.4±4.2Ca	11.6±4.7Ba	0.5±0.2Aa	0.8±0.1Aa
	UNIJ				
	0%	4.1±2.6Aa	10.1±2.6Ba	0.5±0.3Aa	0.8±0.9Aa
	50%	10.9±3.3Ba	9.8±3.0Ba	0.4±0.3Aa	0.4±0.2Aa
	100%	11.5±5.0Ba	8.1±3.9Ba	0.5±0.1Aa	0.6±0.1Aa
	HAB				
	0%	tr	0.1±0.0Aa	tr	
	50%	tr	0.1±0.0Aa	0.1±0.0Aa	
	100%	tr	0.1±0.0Aa	0.1±0.0Aa	
Caps ME					
	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	0.4±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 lower case represents water supply according to Tukey's HSD post hoc test; ND: not detected; tr: traces.

The sum of individual peaks represents the total carotenoid concentration as identified on the chromatogram. A lower concentration of carotenoid compounds was found in the first harvest when compared to the second, third, and fourth 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 water supply treatments in the first harvest, no influence was found in HET, even though a slight decline in concentration was recorded as the water supply 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 water supply increased was found in UNIJ.

Considering the total carotenoid concentration in the third harvest, it decreased as the water supply increased in all cultivars. Nonetheless, in HET and HAB, no significant differences were found in them. Under 100% 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 water supply treatments, even though a slight decline in concentration as water supply increased was evident (fig 14).

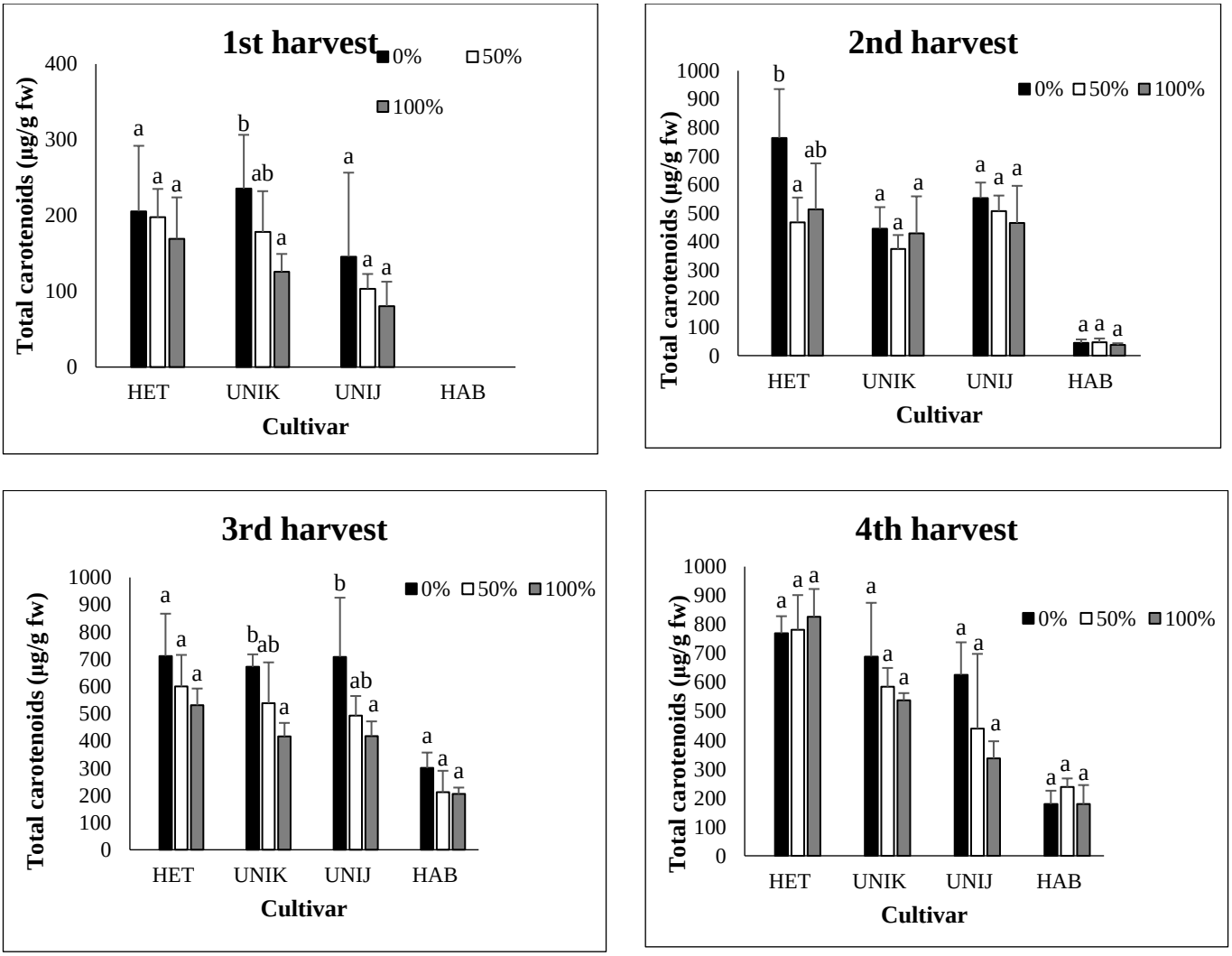


Figure 14. Mean concentration of total carotenoids present in the chilli pepper cultivars at the various harvesting stages in the 2019 growing season.

In this study, the peaks analysed (Free capsanthin, Free zeaxanthin, Capsanthin mono-esters, Zeaxanthin mono-esters, Beta carotene, Capsanthin di-esters, Zeaxanthin di-esters) were characterised depending on their relevance in food chemistry (Table 11, 12). Capsanthin gives the primary red colour of peppers, zeaxanthin represents the yellow colour during ripening, and β-carotene is essential from the nutritional point of view (Arimboor & Natarajan, 2015). A higher concentration of Free caps was found in UNIK peppers (64.6±11.2 µg/g) under non-irrigated condition (0%) in the third harvest and lowered in HAB (0.2±0.0 µg/g) in 2018 (Table 11). By the second growing season, less precipitation caused a decrease in Free caps concentration in HET (2.8±0.5 µg/g) and UNIK (3.5±0.5 µg/g), as well as in HAB (0.1±0.0 µg/g) (Table 12). The higher concentration of Free caps produces red colour and aroma (Deng et al., 2018). A similar trend was

observed in the concentration of Caps ME and Caps DE in both years. Free zeax was higher in UNIK (16.1±3.8 µg/g) under the third harvest (0%) and lower in UNIJ (0.2±0.1 µg/g) under 100% and absent in HAB (Table 11). However, in 2019 Free zeax concentration was higher in HET (15.1±3.5 µg/g) and UNIK (16.1±9.9 µg/g) and lower in HAB when compared to the concentration of HET (4.1±1.5 µg/g), UNIK (5.2±1.41µg/g) and HAB (0.4±0.2 µg/g; not detected) in 2018. Similarly, Zeax ME concentration was higher in UNIK and lower in HAB in both years. However, in other zeaxanthin and capsanthin compounds in the second year (2019), lower concentration were recorded when compared to the first year (2018). The degradation of carotenoid concentration in the second growing season as observed in this study may be as a result of oxygenation which is a major determinant in carotenoid degradation, particularly when pepper fruits become sensitive to light, heat, and oxygen (Carnevale et al., 1980). Based on findings in this study, capsanthin was higher in 0% but did not change in 50% deficit. However, in 100%, a decline in concentration was evident. The concentration of Zeax DE was found to be higher in HET and under detection limit in UNIK and HAB.

According to literature, β-carotene is a crucial component of carotenoids primarily found in vegetables such as red peppers (de Azevedo-Meleiro & Rodriguez-Amaya, 2009). Beta-carotene was higher in HET (74.4±20.8 µg/g), UNIK (84.9±23.9 µg/g), and UNIJ (58.4±13.8 µg/g) but in significantly lower concentration in HAB (0.1±0.0 µg/g) (Table 11). A decline in β-carotene concentration was observed in the second growing season (HET, 30.32±11.33 µg/g; UNIK, 13.6±2.8 µg/g; UNIJ, 18.0±7.1 µg/g and HAB, 0.9±0.8 µg/g) (Table 11). A decrease in β-carotene in the second growing season may be a result of decreased precipitation and water supply treatment which may trigger the presence of P-cryptoxanthin, antheraxanthin, and violaxanthin which contribute to the rapid synthesis of keto xanthophylls during pepper fruit ripening. (Davies et al., 1970; Deli et al., 1992). A higher concentration of total carotenoids was found in HET, UNIK, and UNIJ during the third harvest (0%) and decreased in HAB (Fig 13, 14). However, the lower concentration of carotenoids during fruit ripening remains low, as exhibited by HAB throughout the study (Ha et al., 2007). HAB had yellow-like colour attributes when compared to other cultivars. This is in agreement with a previous study which indicated that total carotenoids are higher in red peppers than in yellow peppers (Ornelas-Paz et al., 2013). Irrespective of the cultivar and harvesting periods, a higher concentration of total carotenoids was found in 0% and 50%. A lower concentration of carotenoid concentration was evident in 100%.

4.4 Phytochemicals in dried/ irradiated peppers

4.4.1 Total Capsaicinoids concentration

Red-ripen, and brick-red peppers were irradiated at a dose rate of 0.5 kGy and 5.0 kGy. 0 kGy was used as a control (Table 13). In HET red peppers, irradiation at a dose rate of 0.5 kGy, NDC, DC, iDC, and HDCs concentration decreased significantly ($p<0.05$) when compared to 0 kGy (control). However, at a dose rate of 5 kGy, capsaicinoids concentration were not influenced when compared to 0 kGy. CAP concentration was not affected by dose application. HCAP was absent. In HET brick-red, irradiation application did not influence NDC, HCAP, and HDCs concentration. The concentration of CAP, DC, iDC, and HDCs was found to be lower at 5.0 kGy. However, in DC, concentration significantly ($p<0.05$) decreased as the dose rate increased. UNIK red decreased in NDC, CAP, DC, and HDCs at a dose rate of 5 kGy, and similarly, in UNIK brick red, a decrease in NDC, CAP, DC, iDC, HCAP, and HDCs concentration was evident at the same dose when compared to 0 kGy. In UNIJ red, irradiation application did not influence major capsaicinoids concentration and other homologue compounds but decreased in HDCs at a dose rate of 5 kGy. Also, in UNIJ brick-red, at a dose rate of 5 kGy, the concentration of NDC, CAP, and HDCs decreased when compared to 0 kGy (Table 13).

Table 13: The effect of irradiation dose treatment on the capsaicinoid composition in red peppers at different ripening stages in the 2018 season. The means are expressed in ± S.D (n = 4) based on dried weight (µg/g).

	Irradiation dose		
Capsaicinoid	**0 kGy**	**0.5 kGy**	**5 kGy**
	HET red		
NDC	301±0.00b	140.0±0.00a	315.0±148.49b
CAP	1890.0±0.00a	994.0±99.00a	1669.5±143.54a
DC	1554.0±0.00b	668.5±54.45a	1438.5±539.52b
HCAP	ND	ND	ND
iDC	189.0±0.00b	87.5±4.95a	168.0±59.40b
HDCs	154±0b	78.4±2a	122.5±44.5b
	HET brick-red		
NDC	350.0±0.00a	332.5±24.75a	245.0±49.50a
CAP	2765.0±0.00b	2432.5±222.74b	1508.5±539.52a
DC	1785.0±0.00c	1729.0±168.29b	1085.0±247.48a
HCAP	91.0±0.00a	98.3±6.43a	76.6±9.40a
iDC	63.0±0.00b	55.3±3.96b	38.8±4.45a
HDCs	154±0a	154±0a	91±10a
	UNIK red		
NDC	98.0±0.00b	108.5±44.55b	73.5±4.95a
CAP	658.0±0.00b	917.0±207.90b	420.0±0.00a
DC	588.0±0.00b	577.5±173.24b	353.5±4.95a
HCAP	227.5±0.00c	17.5±2.97b	16.4±4.45a
iDC	122.5±0.00b	14.0±2.00a	8.0±0.49a

HDCs	58.8±0a	44.1±1a	32.9±5a
	UNIK brick-red		
NDC	280.0±0.00b	210.0±99.00b	150.5±14.84a
CAP	1120.0±0.00b	770.0±99.00b	455.0±49.49a
DC	1127.0±0.00b	787.5±173.24b	539.0±29.70a
HCAP	58.8±0.00b	32.9±8.91a	34.3±3.96a
iDC	25.2±0.00b	14.0±3.96a	10.8±0.50a
HDCs	111.3±0a	82.6±15a	72.4±0.5a
	UNIJ red		
NDC	2450.0±0.00a	2695.0±346.48a	2537.5±123.74a
CAP	32025.0±0.00a	32077.5±5617.96a	29767.5±4925.00a
DC	23450.0±0.00a	25987.5±6310.92a	21595.0±1138.44a
HCAP	637.0±0.00a	609.0±237.58a	586.2±86.62a
iDC	273.0±0.00a	231.0±59.39a	288.7±86.62a
HDCs	210.±0.00a	1015±99c	840±0b
	UNIJ brick-red		
NDC	3500.0±0.00b	3185.0±445.48b	2327.5±321.73a
CAP	36400.0±0.00b	33267.5±2004.65b	24500.0±4454.77a
DC	28770.0±0.00a	22750.0±989.95a	16502.5±4529.02a
HCAP	738.5±0.00a	787.5±74.25a	707.0±39.60a
iDC	378.0±0.00a	262.5±74.24a	253.7±12.37a
HDCs	1260±0b	1015±247.5a	787.5±24.8a

The same letter represents no significant differences ($p<0.05$) at the application of irradiation dose; ND: not detected; NDC: nordihydrocapsaicin; CAP: capsaicin; DC: dihydrocapsaicin; HCAP: homocapsaicin; iDC: dihydrocapsaicin isomer; HDCs: homodihydrocapsaicin.

In the second year, HET, UNIK, and UNIJ, when subjected to 2.5 kGy, 7.5 kGy, and 10 kGy doses, showed a significant effect ($p<0.05$) in NDC, DC, HCAP, iDC, and HDCs (Table 14). At dose 2.5 kGy, a significant increase ($p<0.05$) in NDC was evident in HET and UNIJ samples. It was observed that an irradiation dose of 10 kGy had a significant effect ($p<0.05$) on all compounds (NDC, CAP, DC, HCAP, iDC, and HDCs). Generally, irradiation doses showed no significant effect ($p<0.05$) on the significant components of pungency, capsaicin (CAP); the different capsaicinoids showed a different response to irradiation in different chili cultivars. However, at 7.5 kGy treatment, UNIK was evident on CAP, DC, and iDC.

In all examined chilli cultivars, the highest loss of most capsaicinoids was recorded for the treatment of 10 kGy. It is remarkable that in HET, the content of HCAP and iDC decreased, to a great extent, with irradiation irrespective of the dose applied, and the damage of capsaicinoids was evident even with treatment of 7.5 kGy. The overall damage of the major capsaicinoids caused by irradiation at 10 kGy ranged between 12 and 25% as compared to untreated control samples, while some minor constituents such as HCAP and iDC lost 58-80% of their content as a consequence of irradiation of HET cultivar. As regards total capsaicinoids, the different genotypes differed in the ratio of their content at 0 versus 10 kGy, which was 1.17,1.23 and 1.00 for HET, UNIK, and UNIJ,

respectively indicating that γ-irradiation at high doses may initiate interconversion between capsaicinoid compounds in some genotypes.

Table 14: The effect of irradiation dose treatment on the capsaicinoid composition in red peppers in the 2019 cultivation season. The means are expressed in ± S.D (n = 4) based on dried weight (μg/g).

Capsaicinoid	**Irradiation Dose**			
	0kGy	**2.5kGy**	**7.5kGy**	**10kGy**
		HET		
NDC	159±11b	175±19b	128±8a	120±9a
CAP	1477±124b	1482±81b	1290±52a	1309±39a
DC	893±82b	980±61b	698±53a	772±58a
HCAP	29±1b	6±0.4a	8±1a	6±1a
iDC	47±8c	27±1b	19±3a	20±2a
HDCs	96±11a	82±4a	74±6a	73±9a
Total	2701±237b	2752±170b	2217±123a	2300±118a
		UNIK		
NDC	72±4b	71±1b	82±8b	55±6a
CAP	458±29b	432±12b	579±77c	399±19a
DC	327±20b	331±11b	397±21b	258±36a
HCAP	7±2b	6±1b	6±1b	4±0.4a
iDC	7±1b	11±2c	8±1c	4±1a
HDCs	48±3c	39±3b	35±3b	28±3a
Total	919±59a	890±30a	1107±112b	748±69a
		UNIJ		
NDC	758±73a	1097±53c	817±40b	665±53a
CAP	12542±663a	13895±1156a	12437±525a	12367±1125a
DC	6487±374a	8878±638b	7035±490a	6720±718a
HCAP	54±3a	69±4b	50±18a	50±5a
iDC	81±9b	200±56c	100±27b	58±9a
HDCs	247±5a	304±21b	243±7a	228±16a
Total	20169±1127a	24443±1928b	20682±1107a	20088±1926a

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

Dose application varied in 2018 (0.5 kGy and 5.0 kGy at different ripening stages) (Table 13) and 2019 (2.5 kGy, 7.5 kGy, and 10 kGy on ripened peppers only) (Table 14). In both studies, 0 kGy was used as control. The food industry and its future of meeting potential consumer demands in applying new technologies necessitated our research into dried pepper for irradiation since their new state is highly susceptible to spoilage (Sádecká, 2017).

Irradiation dose decreased both in major capsaicinoids concentration (CAP and DC) and homologue compounds (NDC, iDC, and HDCs) in HET red at a dose rate of 5.0 kGy but in both major capsaicinoids concentration (CAP and DC) and homologue compounds in HET red at a dose

rate of 0.5 kGy, but at 5.0 kGy in HET brick-red, CAP and DC concentration decreased. A decline in pungency in both red and brick-red may not necessarily be a result of the effect of irradiation application. However, a decrease in pungency maybe as a result of oxidation reaction during drying and packaging, which can contribute to a decline in pungency (Contreras-Padilla & Yahia, 1998). At 0 kGy, CAP and DC concentration were high in UNIJ brick red (36400.0±0.00 µg/g), HET brick-red (2765.0±0.00 µg/g) and UNIK brick-red (1120.0±0.00 µg/g) when compared to UNIJ red (32025.0±0.00 µg/g), HET red (1890.0±0.00 µg/g) and UNIK red (658.0±0.00 µg/g) respectively (Table 13).

CAP concentration varied in cultivars at different drying methods (Yaldiz et al., 2010). The various doses (2.5 kGy, 7.5 kGy, and 10 kGy) had a significant effect ($p<0.05$) on NDC, DC, HCAP, iDC, and HDCs. CAP levels were not altered after irradiation, even though a slight increase in pungency was found in UNIJ (14116.7 ± 1457.17 µg/g) at a dose rate of 10 kGy (Table 14). Primarily, the application of irradiation is to sanitise and decontaminate food products and extend their shelf life (Farkas, 1998). A dose rate of a maximum of 10 kGy is not expected to affect capsaicinoid concentration in our study significantly. The low levels of capsaicinoids determined for UNIK and HET hybrids correspond to the low and moderate pungencies found for peppers belonging to Capsicum species (Liu et al., 2013).

4.4.2 Tocopherol / Vitamin E

The major tocopherol concentration (γ-toc, β-toc, and α-toc) were not influenced by irradiation application in HET red and HET brick-red. However, in HET brick-red, the α-toc concentration decreased at a dose rate of 5.0 kGy. An increase in α-toc QH2 was found to be significantly ($p<0.05$) high in HET red at doses 0.5 kGy and 5.0 kGy when compared to 0 kGy (control). A further increase and decrease in γ-toc ester concentration were found in HET red, but in HET brick-red, a decrease in concentration was evident. The other minor tocopherol concentration was not affected.

IN UNIK red and brick-red, γ-toc, β-toc, and α-toc ester, irradiation application did not influence their concentration. A decline in α-toc hydroquinone and β-toc ester concentration was found in UNIK red and brick-red at a dose of 5.0 kGy. However, in UNIK brick-red, the concentration of α-toc hydroquinone, α-toc, and -toc ester increased significantly ($p<0.05$) at a dose rate of 0.5 kGy when compared to 0 kGy (control).

In UNIJ red and brick-red, tocopherol concentration in γ-toc, β-toc and α-toc QH2 were not influenced by the irradiation doses. A significant increase in α-toc concentration was found in UNIJ red and brick-red. β-toc ester and α-toc ester concentration increased in UNIJ red as the dose increased, but in UNIJ brick-red, dose application did not affect their concentration (Table 15).

Table 15: The effect of irradiation dose treatment on tocopherol compounds in red peppers at different ripening stages in the 2018 season. The means are expressed in ± S.D (n = 4) based on dried weight (µg/g).

	Irradiation dose		
Capsaicinoid	**0 kGy**	**0.5 kGy**	**5 kGy**
	HET red		
γ-toc	0.8±0.00a	1.4±0.31a	1.2±0.00a
β-toc	1.2±0.00a	1.2±0.06a	1.1±0.00a
α-toc QH2	38.4±0.00a	58.4±4.79b	50.9±0.00b
α-toc	156.3±0.00a	146.8±5.39a	154.7±0.00a
α-toc ester	3.9±0.00a	3.4±0.72a	3.1±0.00a
	HET brick-red		
γ-toc	0.7±0.00a	0.5±0.12a	0.4±0.00a
β-toc	1.3±0.00a	1.5±0.13a	1.4±0.16a
α-toc QH2	38.7±0.00a	50.6±10.2a	50.0±2.45a
α-toc	223.0±0.00b	164.0±17.4b	127.5±9.93a
α-toc ester	2.5±0.00a	1.6±0.16a	1.4±0.10a
	UNIK red		
γ-toc	0.7±0.00a	0.9±0.08a	0.5±0.03a
β-toc	2.5±0.00a	2.4±0.03a	1.9±0.45a
α-toc QH2	80.8±0.00a	89.4±12.4b	76.2±18.03b
α-toc	224.5±0.00b	215.2±0.97b	162.1±10.62a
α-toc ester	24.2±0.00a	21.4±0.73a	20.5±1.82a
	UNIK brick-red		
γ-toc	0.4±0.00a	0.9±0.50a	0.7±0.11a
β-toc	2.4±0.00a	2.6±1.27a	2.2±0.38a
α-toc QH2	73.7±0.00a	89.5±41.06b	76.3±4.04a
α-toc	110.0±0.00a	189.0±8.7b	190.8±10.46b
α-toc ester	16.2±0.00a	27.9±10.5a	25.0±4.60a
	UNIJ red		
γ-toc	0.4±0.00a	0.4±0.09a	0.3±0.04a
β-toc	0.9±0.00a	0.6±0.04a	0.6±0.08a
α-toc QH2	24.4±0.00a	25.7±2.61a	24.3±0.65a
α-toc	47.1±0.00a	199.6±16.17b	159.8±1.94b
α-toc ester	7.4±0.00b	11.6±2.3a	11.4±0.05a
	UNIJ brick-red		
γ-toc	1.5±0.00a	0.6±0.10a	0.5±0.07a
β-toc	1.1±0.00a	0.6±0.07a	0.6±0.01a
α-toc QH2	30.0±0.00a	32.1±0.50a	25.4±3.64a
α-toc	173.7±0.00a	256.5±20.2b	150.2±20.95a
α-toc ester	14.1±0.00a	18.0±2.39a	12.1±0.84a

The same letter represents no significant differences ($p<0.05$) at the application of irradiation dose; ND: not detected; γ-toc: γ-tocopherol; β-toc: β-tocopherol; α-toc QH2: α-tocopherol hydroquinone; α-toc: α-tocopherol; α-toc ester: α-tocopherol ester.

As the first pattern of tocopherol biosynthesis is the same as that of carotenoids, it is also expected to be promoted by the low dose of irradiation. The results in Table 16 supported the aforementioned concept. γ-irradiation of HET and UNIK at 2.5 and 7.5 kGy increased the level of α-T, α-TES, and total tocopherols, whereas such tendency held not true in UNIJ, in which the content of the major analogues either remained unaffected or decreased with the treatments. The detrimental effect of a 10 kGy dose of irradiation was found only in HET, in which a reduction of about 10% was assessed as a result of irradiation at the high dose. Another interesting change as a function of 10 kGy treatment is the significant increase in the total tocopherol content in UNIK.

The following tocopherol compounds were detected on the chromatogram: α-TQH2: α-hydro tocopherol quinone, α-T: α-Tocopherol, and α-TEs: α-Tocopherol ester (Appendix 4). The content of α-TQH2 in UNIK increased together with α-tocopherol at a low dose, but it did not correlate with the decreased level of α-T at the higher doses applied to different cultivars and showed high stability towards the ionizing energy of γ-irradiation. It is also evident that response to γ-irradiation of tocopherols is cultivar and dose-dependent (Table 16).

Table 16: The effect of irradiation dose treatment on tocopherol compounds in red peppers in the 2019 cultivation season. The means are expressed in ± S.D (n = 3) based on dried weight (µg/g).

Doses/	**Tocopherol analogues**			
Cultivars	**α-TQH2**	**α-T**	**α-TEs**	**Total**
HET				
0kGy	120.6±12.1a	342.0±23.2b	16.9±2.1a	415.0±21.7b
2.5kGy	127.4±16.2a	375.4±14.1b	26.1±4.2b	464.1±30.0c
7.5kGy	151.6±12.6a	373.4±7.6b	26.2±5.0b	472.0±23.0c
10kGy	139.2±12.6a	311.8±4.0a	18.6±1.8a	378.9±12.7a
UNIK				
0kGy	113.1±2.1a	290.0±30.0a	27.7±7.5a	440.9±35.3a
2.5kGy	135.6±4.6b	388.8±36.5b	40.2±4.2b	582.2±46.5b
7.5kGy	150.1±19.3b	384.4±7.5b	41.8±3.8b	594.2±32.3b
10kGy	141.3±12.0b	320.4±16.2a	32.3±1.9a	524.7±36.0b
UNIJ				
0kGy	194.3±5.5b	372.3±13.8b	33.4±2.6a	611.7±23.3b
2.5kGy	151.5±7.6a	346.8±21.4b	28..4±0.9a	536.0±31.5a
7.5kGy	188.2±11.3b	319.7±14.5a	29.3±3.4a	549.5±50.8a
10kGy	199.0±17.5b	366.3±7.1b	31.8±1.3a	608.9±27.1b

The same letter represents no significant differences ($p<0.05$) at the application of irradiation dose; α-TQH2: α-hydrotocopherol quinone, α-T: α-Tocopherol, α-TEs: α-Tocopherol ester. The values represent SD; n=3.

In the previous year (Table 15), the major tocopherol concentration (γ-toc, β-toc, and α-toc) were not affected at a low dose application in HET red, UNIK red, and UNIJ red. However, the minor tocopherol concentration (α-toc ester) in HET brick-red, UNIK brick-red, and UNIJ brick-red were not stable in the entire study. A decrease and increase in tocopherol concentration, mostly minor compounds, were reported in this study. Hassanein et al. (2003) reported that decreased tocopherol concentration maybe as a result of degradation and unsaturated fatty acids peroxidation. It revealed in this study that HET brick-red had a decreased α-toc concentration at 5.0 kGy, an increase at 0.5 kGy in UNIK brick-red, and a decrease at 0.5 kGy in UNIJ brick-red. The concentration of γ-toc in HET brick-red was not significantly different from UNIK brick-red and UNIK brick-red. However, the concentration of α-toc is found in pepper fruit pericarp and γ-toc in seeds, and therefore, an increase in α-toc concentration in dried peppers indicates fewer seeds in fruit (Bosland et al., 2012).

Generally, irradiation did not influence tocopherol concentration HET, UNIK, and UNIJ. (Table 16). However, at a dose rate of 2.5 kGy, UNIJ had reduced α-toc quinone concentration but was not in the case on HET and UNIK. Van Calenberg et al. (1998) reported that application of irradiation resulted in a general increase of α-toc quinone in spices which is not in conformity with the findings of this research. A decline in α-toc quinone and γ-toc ester concentration as the irradiation dose increased in UNIJ. An increased dose decreased potential antioxidants in black peppers (Suhaj et al., 2006), sunflower, and soybean oils (Lalas et al., 2007).

4.4.3 Carotenoids

The quantitative determination, by HPLC, indicated the significant differences between the different chilli cultivars examined in their total carotenoid content in 2018 (Table 17). Free caps, Caps ME, and Caps DE concentration decreased at a dose rate of 0.5 kGy and 5.0 kGy in HET red and UNIK red. UNIJ red had decreased Caps ME at a dose of 5.0kGy. Free caps concentration in HET brick-red and UNIJ brick-red were not influenced by irradiation dose application, but in UNIK brick-red, a decline in concentration was found in 5.0 kGy. Caps ME and Caps DE concentration decreased in HET brick-red, UNIK brick-red, and UNIJ brick-red at a dose rate of 5.0 kGy. The concentration of Zeax ME and Zeax DE decreased at 5.0 kGy. However, Zeax DE concentration was absent in UNIJ. β-carotene concentration decreased in all cultivars at a dose rate of 5.0 kGy, but in UNIK red, irradiation did not influence their concentration.

The sum of all individual peaks identified in dried peppers on the chromatogram represents the total carotenoids concentration. Total carotenoids concentration was found to be significantly

($p<0.05$) high in UNIJ red (0 kGy, 0.5 kGy, and 5.0 kGy) when compared to HET red and UNIK red. A similar trend was found in brick-red of all cultivars. A significant ($p<0.05$) decrease in total carotenoids concentration was found in 5.0 kGy. As the irradiation dose increased, total carotenoids concentration decreased.

Table 17. Effect of gamma irradiation application on carotenoid concentration in red peppers in the 2018 season. The means are expressed in ± S.D (n = 4) based on dried weight (µg/g).

	Irradiation dose		
Carotenoid group	**0 kGy**	**0.5 kGy**	**5 kGy**
	HET red		
Free caps	72.1±0b	20.9±0.6a	17.6±1.6a
Free zeax	7±0a	5.5±2.1a	5.1±1.5a
Caps ME	108.5±0b	71.5±15.3a	50.8±23.5a
Zeax ME	142±0b	67.7±18.1a	57.7±20a
β-carotene	348.6±0b	173.2±44a	201.2±68.9b
Caps DE	434±0a	153.1±10.6b	127.5±90
Zeax DE	292.2±0a	100.9±4.8b	82.6±32.2b
Total	1404.4±0a	1404.4±0a	542.5±237.8b
	HET brick-red		
Free caps	16.6±0a	22.8±3.6a	14.9±0a
Free zeax	5.9±0a	4.4±1.1a	4.3±0.3a
Caps ME	102.5±0a	46.1±2.6b	24.4±4.4c
Zeax ME	183.9±0a	45.9±1.5b	35.7±0.3c
β-carotene	346.7±0a	122.5±7.2b	100.9±0.5b
Caps DE	417.3±0a	145.1±36.9b	47.9±19.2c
Zeax DE	458.2±0a	82.4±9.8b	26.8±0.8c
Total	1531.1±0a	469.2±62.7b	254.9±25.5b
	UNIK red		
Free caps	29.8±0a	19.8±9.4a	13±4.5b
Free zeax	5±0a	5.4±1.7a	6.3±1.6a
Caps ME	99±0a	99.2±40.9b	93.3±16.5b
Zeax ME	254.2±0a	67.9±27.9b	60.7±11.6b
β-carotene	299.9±0a	276.7±106.7a	348.9±43.2a
Caps DE	144.8±0a	201.7±89.6a	150.9±52.6b
Zeax DE	40.1±0a	31.6±4.9a	41.1±0.1a
Total	872.8±0a	702.3±281.1a	714.2±130.1a
	UNIK brick-red		
Free caps	30.7±0a	13.2±4.6b	8.1±1.1b
Free zeax	5.2±0a	4.3±0.2b	3.7±0.3c
Caps ME	110.7±0a	52.6±3.4b	20.7±1.8c
Zeax ME	245.8±0a	70.4±4.5b	34.3±3.6c
β-carotene	357.4±0a	155.3±44.6b	83.7±13.1c
Caps DE	283.6±0a	112.1±14b	106.2±56.6b
Zeax DE	21.9±0a	15±5.3a	6±1.3b
Total	1055.3±0a	422.9±76.6b	262.7±77.8b
	UNIJ red		
Free caps	17.9±0a	15.9±3.4a	14.1±0.6a
Free zeax	5.2±0a	9±0.5a	4.6±0.9a
Caps ME	182.2±0a	176.8±13.4a	97.2±21.0b
Zeax ME	98.6±0a	60.1±0.4b	42.6±2.6c
β-carotene	9±0a	0.8±0.3b	0.8±0.6b

Caps DE	253.5±0a	287.9±0.5a	191.8±24.0a
Zeax DE	ND	ND	ND
Total	566.4±0a	550.5±18.4a	351.1±49.7b
	UNIJ brick-red		
Free caps	5.5±0a	7.6±1.1a	8.1±0.5a
Free zeax	4.2±0a	3.3±0.3a	2.5±0.2a
Caps ME	114.5±0a	103.2±7.3a	56.4±8.2b
Zeax ME	181.5±0a	26.7±4.7b	14.8±0.8c
β-carotene	4.4±0a	2.2±0.1a	0.5±0.1b
Caps DE	124.4±0a	168±4.9b	310.3±27.1c
Zeax DE	ND	ND	ND
Total	434.5±0a	311±18.4a	392.6±36.9a

The same letter represents no significant differences ($p<0.05$) at the application of irradiation dose; ND: not detected; Free caps: Free capsanthin; Free zeax: Free zeaxanthin; Caps ME: Capsanthin mono ester; Zeax ME: Zeaxanthin mono ester; β-carotene: Beta carotene; Caps DE: Capsanthin di-ester; Zeax DE: Zeaxanthin di-ester

The changes that took place on the major groups of carotenoid compounds as a function of irradiation doses during the 2019 season have been summarised (Table 18). A significant increase ($p<0.05$) of most of the carotenoid groups was observed with a 2.5 kGy dose and for some groups with 7.5 kGy treatment in HET and UNIJ, while the significant increase in the total carotenoid content as a result of the low dose treatment was found only in HET. However, the increase of yellow xanthophylls (43%) was significantly higher ($p<0.05$) than that record for red xanthophyll (28%) in the HET cultivar resulting in a substantial decrease in the ratio of red/yellow pigment. An opposite trend was noticed in UNIK, where the R/Y ratio increased with higher doses of irradiation. Unlikely, there was a slight change in R/Y ratio in irradiated samples of UNIJ cultivar except that with 7.5 kGy, which/ was significantly lower ($p<0.05$) than with other treatments confirming the cultivar-and dose-dependence of γ-irradiation effect.

The carotenoids in different cultivars showed different responses to high irradiation doses. The degradation caused by treatment of 10 kGy was evident for all carotenoid groups except diesters of yellow xanthophylls, which exhibited high stability in HET and UNIK, but not in UNIJ, in which degradation of 34, 37, and 38% were recorded for total yellow, total red and total carotenoids respectively were recorded. Such magnitude of impairment is highly significant ($p<0.01$) as compared to 12-18% for the same groups in HET and UNIJ.

In HET, irradiation caused a significant increase ($p<0.01$) in the ratio for both red and yellow xanthophylls irrespective of the applied dose, while in UNIJ, the highest significant increase ($p< 0.05$) was found with the highest doses. As for UNIK, no change was observed in the ratio for both yellow and red xanthophylls.

β-carotene, the primary precursor of vitamin A in peppers, responded to irradiation in different ways depending on the genotype of chili peppers. Its content significantly increased with the dose of 2.5 and 7.5 kGy in HET and UNIJ, while in UNIK, a significant increase was observed with only 2.5 kGy treatment.

Zeaxanthin showed a similar response to that of β-carotene toward low irradiation dose in different cultivars. Nonetheless, it behaved differently with higher dose treatment. No significant changes were found for total zeaxanthin in peppers treated at 10 kGy as compared to the untreated ones in all of the examined cultivars.

Table 18. Change in content (µg/g) of the main carotenoid groups as a function of irradiation dose in the year 2019. The means are expressed in ± S.D (n = 4) based on dried weight.

Carotenoid group	**Irradiation dose**			
	0kGy	**2.5kGy**	**7.5kGy**	**10kGy**
		HET		
Free Yellow Xanthophylls	147.8±9.3a	175.2±5.9b	142.0±5.8c	123.8±10.0a
Yellow MEs	299.3±31.3a	423.6±62.0b	396.9±25.6b	345.3±58.3b
Yellow DEs	101.5±6.8a	164.2±8.2b	141.6±8.9c	106.2±3.9a
Total esterified Yellow	400.8±38.1a	587.8±70.2b	538.5±34.5b	451.6±62.2a
Esterified/Free Yellow	2.7±0.2a	3.6±0.07b	3.8±0.2b	3.7±0.38b
Total Yellow xanthophylls	707.4±60.3a	1014.4±79.0b	984.2±34.7b	712.2±153.9a
Unesterified Red	160.9±11.9a	154.8±8.7a	115.6±10.0b	111.8±15.2b
Red MEs	511.9±32.4a	633.0±21.2b	548.2±51.1a	474.4±27.9a
Red DEs	1316.9±118.1a	1693.2±104.2b	1441.2±132.5a	1118.8±43.0c
Total esterified red	1477.8±150.5a	2326.2±125.4b	1989.4±217.3a	1593.6±70.9a
Esterified/Free Red	9.18±0.81a	15.0±1.8b	17.21±1.7b	14.3±1.3c
Total Red xanthophylls	1984.6±148.3a	2497.3±117.2b	2137.9±221.9b	1720.2±74.3a
β-carotene	156.7±17.9a	251.5±39.7b	303.7±46.1b	190.0±26.7a
Total Zeaxanthin	112.4±4.8a	179.6±8.2b	116.4±18.5a	115.7±13.0a
Total Carotenoids	2692.1±208.3a	3511.8±165.2b	3122.1±188.2b	2432.4±155.8a
Red/yellow	2.80±0.04a	2.47±0.18b	2.17±0.30b	2.50±0.57b
		UNIK		
Free Yellow xanthophylls	160.0±12.2a	138.3±11.9b	134.9±11.0b	139.7±8.2b
Yellow MEs	551.1±33.7a	500.5±27.4a	423.2±34.9b	426.8±36.9b
Yellow DEs	65.0±5.5a	78.7±2.2b	72.9±6.3b	81.1±7.3b
Total esterified Yellow	616.1±39.3a	579.2±29.6a	496.1±41.2b	507.9±44.2b
Esterified/Free Yellow	3.8±0.27a	4.2±0.34a	3.7±0.33a	3.6±0.27a
Total Yellow xanthophylls	776.2±28.5a	707.2±25.9b	634.8±67.6b	682.6±59.4b
Free Red Xanthophylls	249.3±4.6a	205.8±11.4b	217.0±22.9b	211.2±4.7b
Red MEs	333.2±24.6a	308.3±9.1a	316.2±24.5a	254.0±29.7b
Red DEs	1041.5±79.4a	982.9±61.4a	998.3±98.7a	859.8±88.4a
Total esterified red	1374.7±103.9a	1291.2±70.5a	1314.5±123.2a	1113.8±118.1a
Esterified/Free Red	5.5±0.28a	6.3±0.50a	6.1±0.60a	5.3±0.32a

Total Red xanthophylls	1624.1±103.1a	1496.9±25.3a	1525.71±121.0a	1343.6±120.9a
β-carotene	211.4±13.6a	258.3±8.8b	189.3±32.3a	231.2±12.6a
Total Zeaxanthin	257.0±18.9a	336.8±36.6b	294.9±15.6b	206.3±11.6c
Total Carotenoids	4024.4±229.4a	3711.4±304.4a	3734.03±301.2a	3396.0±260.6a
Red/yellow	2.09±0.08a	2.05±0.09a	2.40±0.12b	1.96±0.07a
		UNIJ		
Free Yellow xanthophylls	263.4±23.4a	247.2±28.5a	211.8±23.0a	134.0±10.1b
Yellow MEs	406.1±29.4a	408.4±24.4a	368.3±3.0b	235.2±16.0c
Yellow DEs	197.8±31.0a	278.9±5.2b	275.8±27.4b	197.2±20.9a
Total esterified Yellow	603.9±60.4a	687.3±29.6a	644.1±30.4a	432.4±36.9b
Esterified/Free Yellow	2.3±0.21a	2.8±0.22b	3.0±0.23b	3.2±0.24b
Total Yellow Xanthophylls	1231.3±81.1a	1389.8±29.8b	1280.3±27.0a	819.7±41.5c
Free Red xanthophylls	436.7±37.9a	345.1±24.6b	216.9±381.9c	134.3±34.9d
Red MEs	659.9±83.9a	750.9±55.2b	604.4±55.4a	397.3±9.1c
Red DEs	1314.1±115.6a	1508.3±59.8b	1371.0±50.8a	975.9±90.8c
Total esterified red	1974.0±199.5a	2259.2±115.0b	1975.4±106.2a	1373.2±99.8c
Esterified/Free Red	4.5±0.45a	6.5±0.40b	9.1±1.0c	10.2±1.6c
Total Red xanthophylls	2410.7±129.2a	2604.4±93.3a	2192.4±112.7a	1507.5±47.9b
β-carotene	349.0±22.4a	442.6±41.4b	421.1±37.1b	255.1±13.9c
Total Zeaxanthin	138.4±19.2a	148.4±11.1a	133.0±15.2a	150.5±14.1a
Total Carotenoids	3641.9±209.4a	3875.3±107.7a	3472.6±124.1a	2327.2±88.1b
Red/yellow	1.96±0.08a	1.88±0.06a	1.71±0.08b	1.84±0.04a

Capsanthin concentration (Free caps, Caps ME, and Caps DE) decreased at a dose rate of 0.5 kGy (HET red and UNIK red) and 5.0 kGy (HET red, UNIK red, HET brick-red, UNIK brick-red, and UNIK brick-red) (Table 17). The quantitative determination, by HPLC, indicated the significant differences between the different chilli cultivars examined in their total carotenoid content (Table 18). Capsanthin concentration, the main carotenoids responsible for the red colour attribute in red peppers (Schweiggert et al., 2007), was found to have decreased in the study upon application of irradiation. Degradation of capsanthin in brick-red peppers (Table 17) was evident at a dose rate of 5.0 kGy despite their colour break attributes. Zeaxanthin concentration (Free zeax, Zeax ME, and Zeax DE) at all levels of irradiation application was not stable. In HET red and UNIK red, Zeax ME and Zeax DE concentration decreased at a dose a rate of 5.0 kGy and no effect from irradiation application in HET, UNIK, and UNIJ. Zeaxanthin contributes to a major pigmentation in *Capsicum annuum* even though their concentration is low to significantly influence total carotenoids concentration (Minguez-Mosquera & Hornero-Mendez, 1994). Based on the finding in this study, β-carotene in HET was stable regardless of irradiation application. At a dose of 5.0 kGy and 10 kGy in UNIK and UNIJ, respectively, a significant decrease in β-carotene was found. In both study periods, total carotenoids concentration decreased significantly as irradiation dose increased, which confirms a finding by Topuz and Ozdemir (2003) that in irradiated dried peppers, decreased carotenoids concentration is expected. Carotenoid pigment degradation in red-pepper

powders usually occurs during processing (Kim et al., 2004). It is important to highlight the changes in the ratio of esterified to unesterified carotenoids because esterification with fatty acids increases their stability towards oxidative damage (Biacs et al., 1992). In all cultivars examined, this ratio was significantly higher (3-4 times) with red xanthophylls than with yellow ones (Table 18). Such property is considered as an advantage for red colour intensity and stability at post-harvest. The different cultivars varied substantially in the response of the ratio of esterified to free pigments to irradiation.

It is worthy of mentioning that although carotenoids are somewhat sensitive to ionizing energy, not all groups were susceptible to a dose of 10 kGy. This agrees with the finding of Iqbal et al. (2016) that the damage observed on less stable carotenoids may be similar or less than what happens with thermal processing.

5.0 CONCLUSIONS AND RECOMMENDATIONS

Water supply to plants is a necessary component that contributes to crop growth and yield. Deficit irrigation may be beneficial in areas with water scarcity or shortage. As focused on this study, a continuous harvest of peppers is encouraged, but deficit irrigation should be discontinued under open field environments as the weather conditions change to low temperatures. Maintaining a no irrigation programme (0% water except natural precipitation and fertigation) around these periods would produce high yields in chilli peppers, vitamin C, increase capsaicin in Hetényi Parázs' (HET) and α-tocopherol concentration in 'Hetényi Parázs' (HET) and 'Unikal' (UNIK). Tocopherols decreased as precipitation and irrigation decreased. However, 'Unijol' (UNIJ) may be cultivated for tocopherols (vitamin E) under deficit irrigation and 0% water for yields. Habanero (HAB) under 0% or control under low temperature would be suitable for yield or marketability.

Cultivation of hot or spicy peppers under uncontrolled environmental conditions can affect their growth rate and phytochemical concentration. Elevated temperature, high light exposure, and heat stress result in reduced leaf stomatal conductance. Managing a good irrigation practice for pepper cultivation under an open field environment is achievable. However, the selection of genotype for breeding should consider pepper crops that can withstand a low water environment and not affect their antioxidant composition.

Based on our findings on pungency stability under low irrigation application, UNIJ (Unijol) hybrid pepper and HAB (Habanero) are recommended for consumer preference and pharmacological purposes. Since water stress and optimum water supply may influence poor fruit setting and low yield, HET (Hetényi Parázs) and UNIK (Unikal) hybrid peppers are suitable marketable yield and consumer preference. The present investigation provided new data on the composition and content of essential phytonutrients in new hybrids that may determine their convenient use for human nutrition or industrial applications. The application of gamma irradiation to food as a necessary processing method to avoid post-harvest losses is recommended for future demand to complement food security issues. The favourable effect of a relatively low dose (2.5 kGy) of γ-irradiation before over-ripening opened new possibilities to facilitate the over-ripeness process and improve quality and nutritional attributes of chilli peppers in addition to its advantage in reduction of microbial growth. Furthermore, the application of 10 kGy, the allowed dose for the de-toxification of spice peppers, degrades, to some but not to a great extent, the capsaicinoids and some carotenoids that make it preferred processing to produce safe products for human

consumption with acceptable quality. However, better processing methods to avoid degradation in phytochemicals in irradiated peppers are recommended.

It is further recommended for future studies into the phytochemical response of these pepper cultivars to water supply treatment under modified atmosphere or greenhouses.

6.0 NEW SCIENTIFIC RESULTS

1. I found out that at water deficiency of 50% increased significantly the yield of all chilli pepper cultivar studied, while the optimal water supply decreased the yield up to 52% for Habanero, 5% for 'Unikal', 7% for 'Hetényi Parázs' and 14% for ‘Unijol’;
2. Less water supply increased pungency in 'Hetényi Parázs' and 'Unikal', but more water slightly increased capsaicin in ‘Unijol’ and ‘Habanero’ which are initially very pungent peppers;
3. Also, it was proven that the concentration of tocopherols and carotenoids decreases as accumulated precipitation and irrigation decreased.
4. With Gamma irradiation at the beginning of the over-ripening of the new chilli cultivars, a novel technology was achieved to improve the quality and safety attributes of the spice chilli crop. The novel technology development resulted in some new approaches:
    a. The application of ϒ-irradiation at 2.5 kGy doses improved the concentration of health promotive phytonutrients significantly, thereby increasing the nutritive value and stability of chilli pepper products,
    b. The 10 kGy dose, which is effective in detoxification via retarding the microbial growth, caused degradation to carotenoids and capsaicinoids, but not to a great extent. The maximum loss of 32% for carotenoids and 38% for minor capsaicinoids in the ‘Unijol’ cultivar, while slight degradation occurred in 'Hetényi Parázs' and ‘Unikal’.
5. The use of ripening dynamics on irradiated peppers proved high capsaicinoid concentration in brick-red than in red;
6. α-tocopherol in dried and irradiated pepper cultivars was found to be high when compared to fresh ones.

7.0 SUMMARY

The **Literature review** focused on the global importance of chilli pepper and its recent demand in high quantities. The performance of chilli pepper in Hungary, Africa precisely Ghana was also elaborated. The cultivation technologies in peppers, as well as their response to physiological and biotic factors, was discussed. Water supply, which is an integral part of chilli pepper cultivation, was well discussed and how irrigation treatments can affect or improve their phytochemicals. Phytochemicals focused on total capsaicinoids concentration, vitamin C, Tocopherols (vitamin E), and total carotenoids concentration. However, polyphenols, phenols, and flavonoids were briefly discussed. Fresh peppers, as a highly susceptible crop, were further discussed on how a food processing method such as the application of gamma irradiation can improve their shelf-life without compromising on the phytochemical concentration. In the last section, detailed information on the use of gamma irradiation to extend the shelf-life of dried peppers was discussed.

The **Materials and Methods** focused on a detailed description of the experiment. The study was conducted under open field conditions to investigate the effect of physiological factors and phytochemical responses of chilli pepper cultivars under three different water supply treatments. The study further investigated the effect of dried irradiated peppers on phytochemical responses. The irradiation doses were 0.5 kGy, 5 kGy, 2.5 kGy, 7.5 kGy, 10 kGy, and 0 kGy used as control. The response of pepper cultivars' yield to the water supply was also considered. The experiment was carried out in 2018 and 2019 on a one-hectare plot of land under a well-maintained soil condition.

The experimental design used in both study periods was a randomised complete block (RCBD) with four replications for each water supply treatment. The chilli 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. The water supply treatment was 0% or control (taking into consideration natural precipitation), 50% deficit irrigation, and 100% optimum water supply administered through a drip system. Trends of daily maximum and minimum precipitation and irrigation varied between 340 mm – 620 mm in 2018 and 125 mm – 410 mm in 2019, at an average air temperature of 23.8℃ in 2018 and 24.8℃ in 2019. Under four separate harvests (between August and October for each growing season), peppers were evaluated for vitamin C content, capsaicinoid concentration, tocopherols (vitamin E), and carotenoid concentration using high-performance liquid chromatography (HPLC).

The **Results and Discussion** focused on the objectives of the study. In the field measurements of physiological parameters during the growing seasons, water supply treatments significantly influence ($p<0.05$) physiological factors (canopy temperature, Fv/Fm, and soil moisture). In the various pepper cultivars, HAB responded poorly to the effect of water supply treatments on physiological factors. HET and UNIJ were very stable throughout the open field experiment.

The marketable yield measured at their fresh base weight, depending on water supply treatments, 0% and 50%, produced a higher quantity in the second harvest. HET, UNIK, and UNIJ produced marketable yield in the second harvest, in HAB during the third harvest, and in minimum quantity for the subsequent harvest. Pepper cultivars responded well to yield under non-irrigated and 50% deficit. Total marketable yield for the second year; HET (24.67±3.34 t/ha), UNIK (13.24±1.44 t/ha), UNIJ (20.45±3.89 t/ha), and HAB (15.04±1.56 t/ha) were significantly higher when compared to the first year; HET (16.62±0.94 t/ha), UNIK (19.67±1.22 t/ha), UNIJ (12.8±1.00 t/ha) and HAB (7.92±0.79 t/ha). Accumulated precipitation and irrigation in 2019 was suitable for higher yields in 0% and 50%. However, a decreased temperature by the fourth harvest decreased yields. The temperature under the open field conditions prior to the autumn frost contributed to a decline in yield by the fourth harvest in both years.

Based on the phytochemical response of fresh peppers to water supply treatments, capsaicinoid concentration was higher in UNIJ, HET, and HAB under non-irrigated conditions and 50% deficit and also lower concentration in UNIK. A lower level of pungency was recorded in all cultivars when they were given optimum water supply. Depending on the effect of harvesting periods on pungency concentration, high amounts were found in the second and third harvests. The major capsaicinoids concentration (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) was high in the second growing season when compared to the first year. However, in both years, the homologue compounds (homocapsaicin, dihydrocapsaicin isomer, homodihydrocapsaicins) were found in minimal quantities. Capsaicin was high in HET in 2019 (584.1±19.1 µg/g) and lower in 2018 (310.1±46.3 µg/g) under 0% or control and 50% deficit irrigation conditions. In contrast, in the case of UNIJ and HAB, capsaicin was lower in 2019 (UNIJ, 1936.3±216.5 µg/g; HAB, 2549.7±81.0 µg/g) and high in 2018 (UNIJ, 6518.7±764.5 µg/g; HAB, 3564.7±150.2 µg/g).

Vitamin C content was found in all harvests for both years. Between water supply treatments, higher amounts were recorded in 0% and 50%. A lower vitamin C content was found in optimum water. HET, UNIK, and UNIJ had higher amounts of vitamin C whereas lower amounts were found in HAB (varied between 596.8±134.6 µg/g – 636.2±69.1 µg/g) in the first year. However, HAB had higher vitamin C in the second year, which varied between 2870.0±148.8 µg/g –

3223.7±118.6 µg/g. Vitamin C content was higher in 2019 than in 2018, under 0% and 50% and lowered in 100%. This indicated that accumulated precipitation and irrigation in 2019 was more optimal for vitamin C content.

All the cultivars had an influence on the major compounds Tocopherols (vitamin E), γ-tocopherol, α-tocopherol, and β-tocopherol. Lower concentrations of γ-toc and β-toc were observed in all cultivars irrespective of the water supply treatments, UNIK (2.2±0.5 µg/g) and HET (2.0±2.3 µg/g) recorded concentration of β-toc under 0% and 50%. Also, α-toc concentration was significantly lower in 100%. The concentration of α-toc under 0% was found to be higher in HET (between 76.6±6.2 µg/g – 81.4±4.6 µg/g) and lower in HAB (between 7.10±4.8 µg/g – 10.4±1.1 µg/g) during the first growing season. However, by the second growing season, a decrease in tocopherol concentration was observed in all cultivars (HET, UNIK, UNIJ, and HAB). A lower concentration of γ-toc was evident in all cultivars irrespective of the water supply treatments. The concentration of α-toc was found to be higher in 0% in HET (between 58.1±7.1 µg/g – 69.0±1.7 µg/g) and lower in HAB (between 1.7±0.8 µg/g – 0.1±0.0 µg/g). β-toc was absent or in minimal amounts in all cultivars.

Other tocopherol concentration was found to be lower, especially under optimum water in both study periods. However, tocopherol concentration was found to be higher in the first growing season when compared to the second year. It was observed in this study that tocopherol concentration decreased as accumulated precipitation and irrigation increased, and therefore, under a moderate temperature, tocopherol concentration may increase. Based on water supply treatments, 50% had minimal influence on tocopherol concentration. This indicates that 50% may not be relevant for tocopherols (vitamin E) since it did not increase or decrease tocopherol concentration. However, maintaining no irrigation (an exception to natural precipitation), tocopherol concentration may increase or stabilise.

In the case of total carotenoids concentration, the analysed peaks (Free capsanthin, Free zeaxanthin, Capsanthin mono-esters, Zeaxanthin mono-esters, Beta carotene, Capsanthin di-esters, Zeaxanthin di-esters) were characterised depending on their relevance in food chemistry. Capsanthin gives the primary red colour of peppers, zeaxanthin represents the yellow colour during ripening, and β-carotene is essential from the nutritional point of view. A higher concentration of Free caps was found in UNIK peppers (64.6±11.2 µg/g) under the non-irrigated condition in the third harvest and lower in HAB (0.2±0.0 µg/g) in 2018. By the second growing season, Free caps concentration decreased in HET (2.8±0.5 µg/g) and UNIK (3.5±0.5 µg/g), as well as in HAB (0.1±0.0 µg/g). A similar trend was observed in the concentration of Caps ME and Caps DE in

both years. Free zeax was higher in UNIK (16.1±3.8 µg/g) under the third harvest (0%) and lower in UNIJ (0.2±0.06 µg/g) under 100% and absent in HAB. However, in 2019 Free zeax concentration was higher in HET (15.08±3.5 µg/g) and UNIK (16.08±9.93 µg/g) and lower in HAB when compared to the concentration of HET (4.1±1.5 µg/g), UNIK (5.2±1.4 µg/g) and HAB (0.37±0.17 µg/g; not detected) in 2018. Similarly, Zeax ME concentration was higher in UNIK and lower in HAB in both years. However, in other zeaxanthin and capsanthin compounds, concentration in the second year (2019) was found to be significantly lower when compared to the first year (2018).

Based on findings in this study, capsanthin was higher in 0% but did not change in 50%. However, in 100%, a decline in concentration was evident. The concentration of Zeax DE was found to be higher in HET and under detection limit in UNIK and HAB. Beta-carotene was higher in HET (74.4±20.8 µg/g), UNIK (84.9±23.9 µg/g), and UNIJ (58.4±13.8 µg/g) but in significantly lower concentration in HAB (0.1±0.0 µg/g). A decline in β-carotene concentration was observed in the second growing season (HET, 30.3±11.3 µg/g; UNIK, 13.6±2.8 µg/g; UNIJ, 18.0±7.1 µg/g and HAB, 0.9±0.8 µg/g). A decrease in β-carotene in the second growing season may be a result of decreased precipitation and irrigation or water supply treatment. Total carotenoid concentrations were found to be higher in HET, UNIK, and UNIJ during the third harvest (0%) and decreased in HAB. Irrespective of the cultivar and harvesting periods, a higher concentration of total carotenoids was found in 0% and 50%. A lower concentration of carotenoid concentration was evident in 100%. It is very necessary to take into consideration that reducing water supply treatments could maintain carotenoids concentration without decreasing quality parameters.

Based on the effect of irradiation application on phytochemicals, the various doses were used; 0.5 kGy and 5.0 kGy at different ripening stages in 2018 and 2.5 kGy, 7.5 kGy, and 10 kGy in 2019. In both studies, 0 kGy was used as control. Irradiation dose decreased in both major capsaicinoids concentration (CAP and DC) and homologue compounds (NDC, iDC, and HDCs) in HET red at a dose rate of 5.0 kGy, in both major capsaicinoids concentration (CAP and DC) and homologue compounds in HET red at a dose rate of 0.5 kGy, and at 5.0 kGy in HET brick-red. At 0 kGy, CAP and DC concentration were high in UNIJ brick red (36400.0±0.0 µg/g), HET brick-red (2765.0±0.0 µg/g) and UNIK brick-red (1120.0±0.0 µg/g) when compared to UNIJ red (32025.0±0.0 µg/g), HET red (1890.0±0.0 µg/g) and UNIK red (658.0±0.0 µg/g) respectively. The various doses (2.5 kGy, 7.5 kGy, and 10 kGy) had a significant effect ($p<0.05$) on NDC, DC, HCAP, iDC, and HDCs. CAP levels were not altered after irradiation, even though a slight increase in pungency was found in UNIJ (14116.7 ± 1457.2 µg/g) at a dose rate of 10 kGy.

On tocopherols in the previous year, the major tocopherol concentration (γ-toc, β-toc, and α-toc) were not affected by low dose irradiation in HET red, UNIK red, and UNIJ red. However, the minor tocopherol concentration (γ-toc ester, β-toc ester, and α-toc ester) in HET brick-red, UNIK brick-red, and UNIJ brick-red were not stable in the entire study. A decrease and increase in tocopherol concentration, mostly minor compounds, were reported in this study. It revealed in this study that HET brick-red had a decreased α-toc concentration at 5.0 kGy, an increase at 0.5 kGy in UNIK brick-red, and a decrease at 0.5 kGy in UNIJ brick-red. The concentration of γ-toc in HET brick-red was not significantly different from UNIK brick-red and UNIK brick-red. Generally, irradiation did not influence tocopherol concentration HET, UNIK, and UNIJ. However, at a dose rate of 2.5 kGy, UNIJ had reduced α-toc quinone concentration but was not in the case on HET and UNIK. A decline in α-toc quinone and γ-toc ester concentration as the irradiation dose increased was found in UNIJ.

On carotenoids, capsanthin concentration (Free caps, Caps ME, and Caps DE) decreased at a dose rate of 0.5 kGy (HET red and UNIK red) and 5.0 kGy (HET red, UNIK red, HET brick-red, UNIK brick-red, and UNIK brick-red). A further decline in Free caps concentration was present in HET, UNIK, and UNIJ at doses 7.5 kGy and 10 kGy. Capsanthin concentration was found to have decreased in the study upon application of irradiation. Degradation of capsanthin in brick-red peppers was evident at a dose rate of 5.0 kGy despite their colour break attributes. Zeaxanthin concentration (Free zeax, Zeax ME, and Zeax DE) at all levels of irradiation application was not stable. In HET red and UNIK red, Zeax ME, and Zeax DE concentration decreased at a dose at a rate of 5.0 kGy and no effect from irradiation application in HET, UNIK, and UNIJ. Based on the finding in this study, β-carotene in HET was stable regardless of irradiation application. At a dose of 5.0 kGy and 10 kGy in UNIK and UNIJ, respectively, a significant decrease in β-carotene was found. In both study periods, total carotenoids concentration decreased significantly as irradiation dose increased.

8.0 APPENDICES

A1: Bibliography

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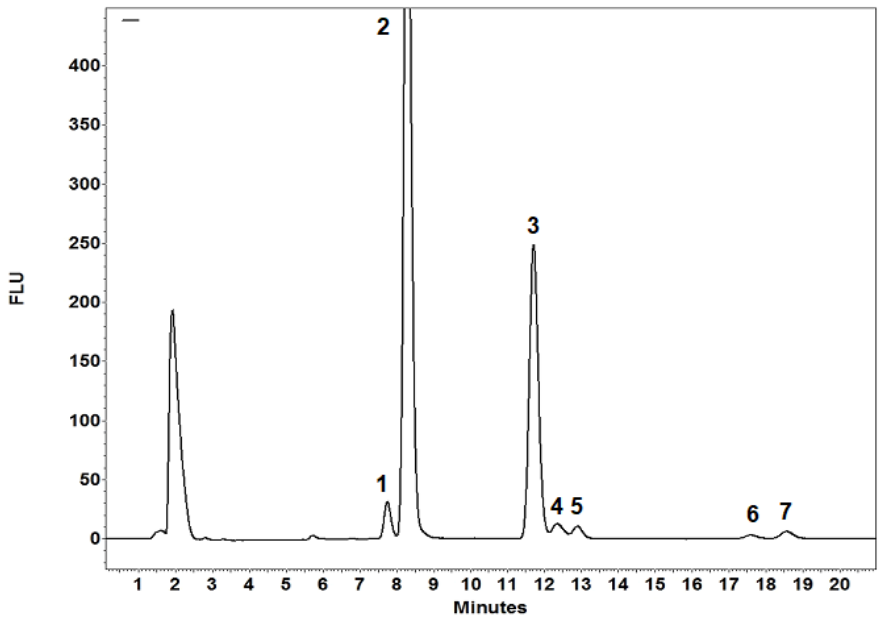
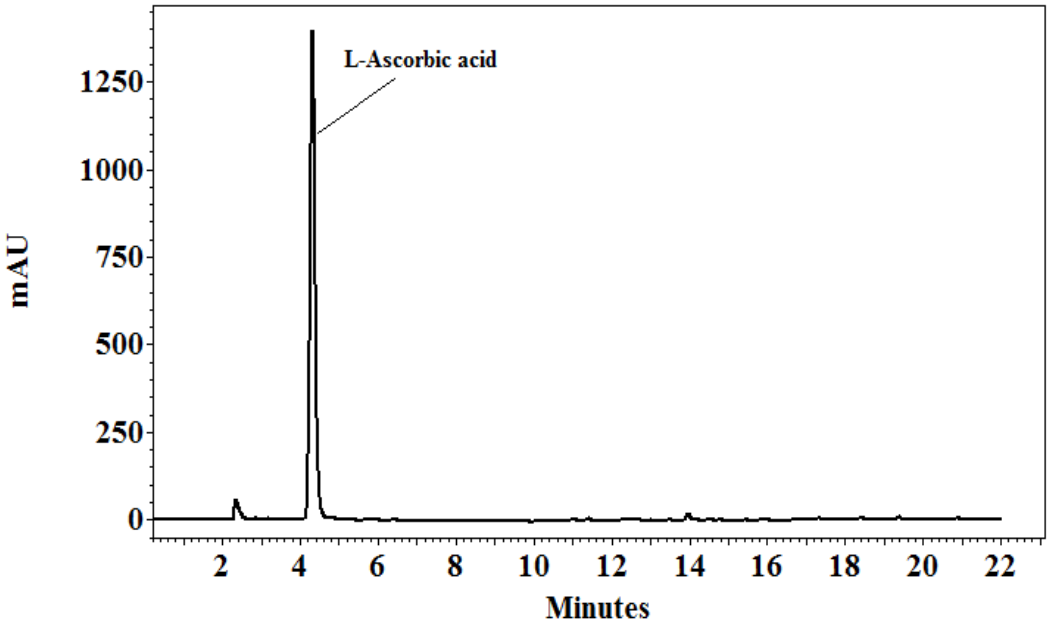
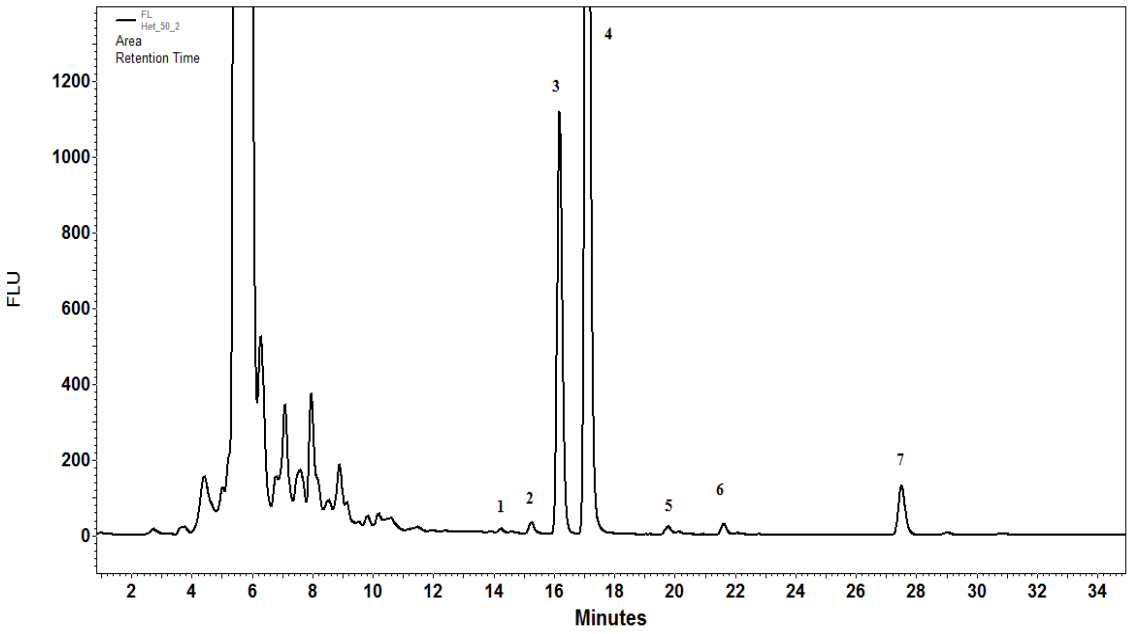
A2: Further appendices



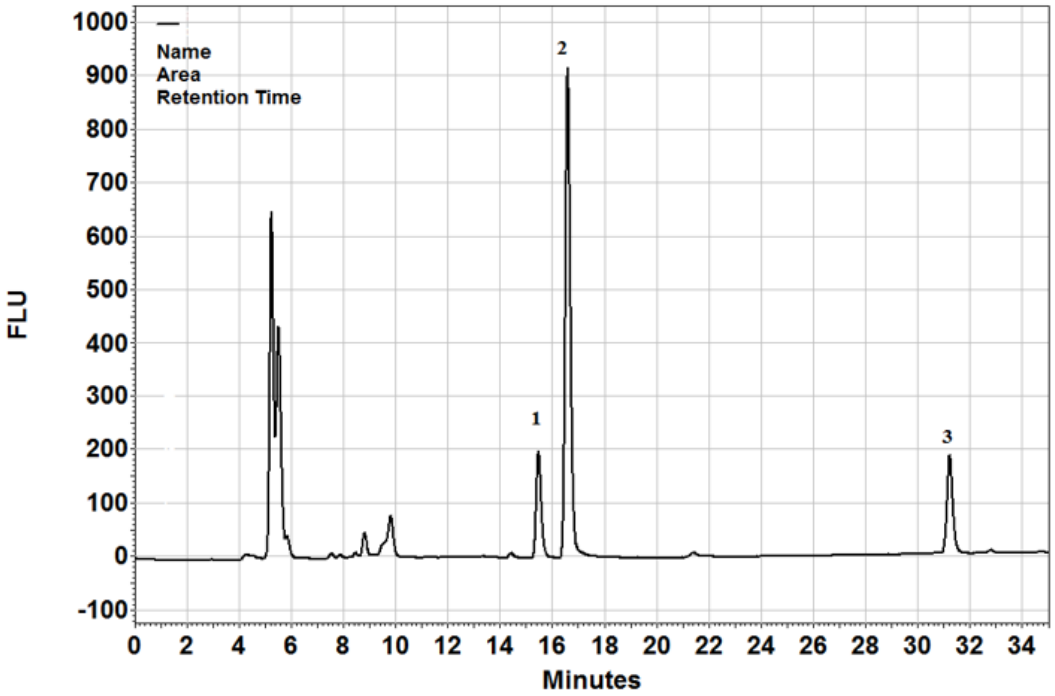
Appendix 1. HPLC profile of chilli capsaicinoids separated on Purospher Star, 3u, 150 x 4.6 mm column with 52:48 acetonitrile-water. The compounds were detected by FL detector at EX:280 and Em: 290 nm. Peak identification: 1: NDC; 2: CAP; 3: DC; 4: iDC; 5: HCAP; 6: HDCs.



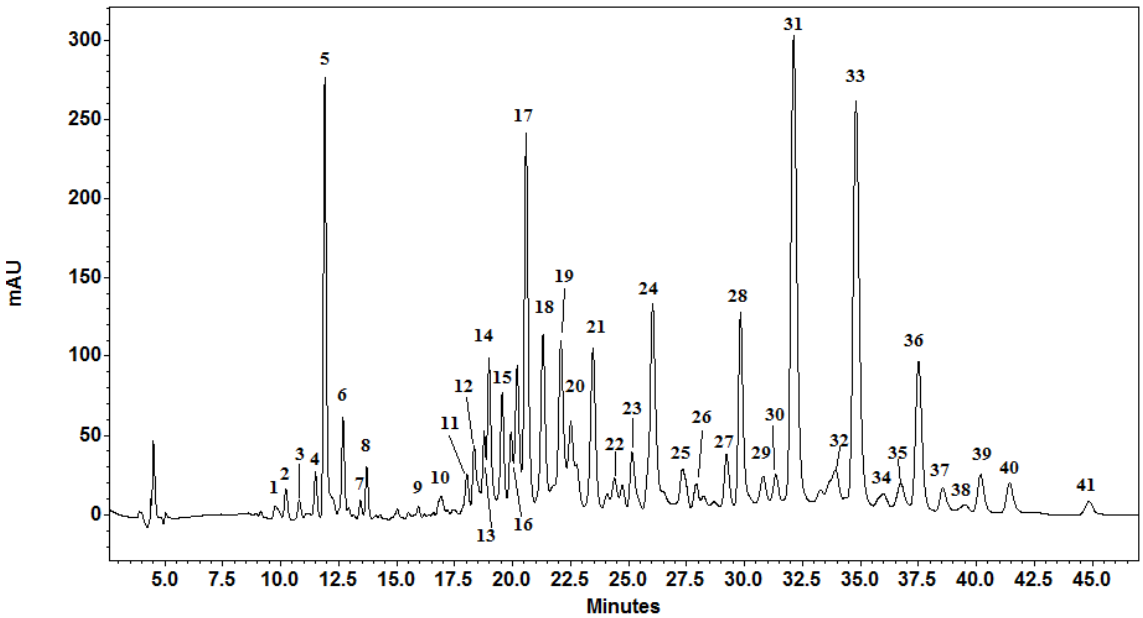
Appendix 2. HPLC profile of L-ascorbic acid separated on Aqua C18, 3u, 150 x 0.46 mm column with gradient elution of (B) Acetonitrile in (A) 0.01M KH_2PO_4 buffer and DAD detection at 265nm. Identification is shown in Table 7. For more details, see text.



Appendix 3. HPLC profile of chilli 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.



Appendix 4. HPLC profile of irradiated samples chilli 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. For more details, see text. Peak identified as α-TQH2, α-T, α-TEs. For more details, see text.



Appendix 5. 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., For more details on peak identification, see text.

Appendix 6. Data used for the identification of carotenoid compounds extracted from red chilli pepper and analyzed by LC-DAD-MS procedure as described in the text.

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

Cultivars studied in this research:

'Hetényi Parázs' (HET), 'Unikal' (UNIK), 'Unijol' (UNIJ), and Habanero (HAB)

HET: *Capsicum frutescens*

UNIK: *Capsicum annuum*

UNIJ: *Capsicum chinense* X *Capsicum annuum*

HAB: *Capsicum chinense*

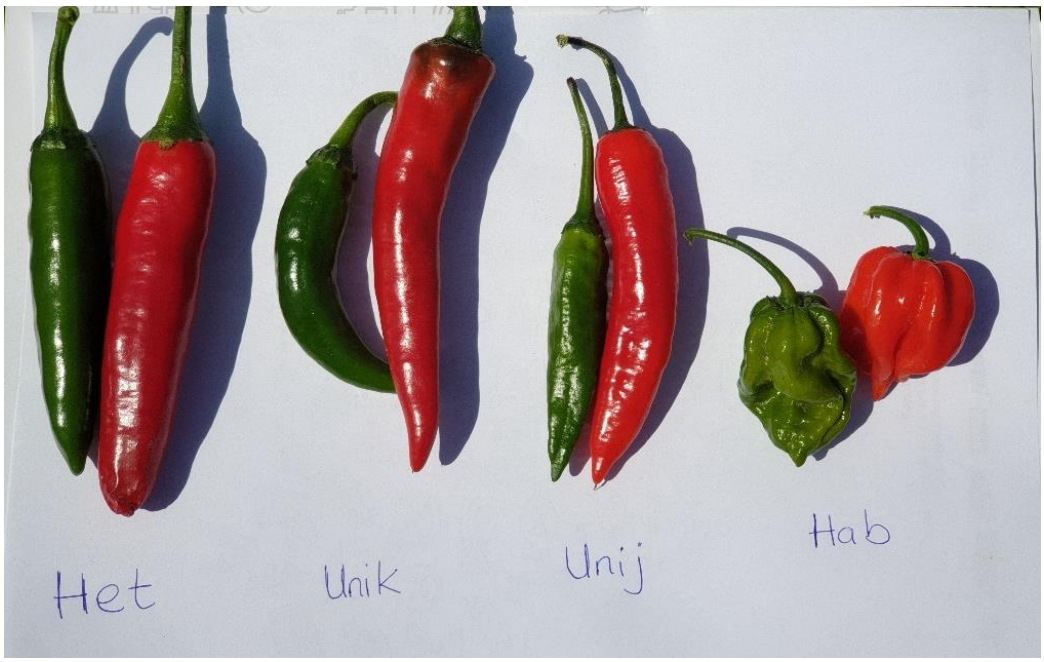


Photo credit: Stella Agyemang Duah

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