Accumulation of α-tocopherol in *Capsicum annuum* L. influenced by changes in water temperature

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A temperature below or above the optimum can reduce the rate of photosynthesis in higher plants, which in turn decreases metabolite precursor pools for the biosynthesis of secondary metabolites. The aim of this study was to determine the effects of seasonal changes in water temperature on α-tocopherol accumulation in pepper (*Capsicum annuum* L.) fruits from an aquaponic system. A portable dissolved oxygen meter was used to measure water temperature and high-performance liquid chromatography was used to determine the levels of α-tocopherol accumulation. Temperature of the fish water ranged from 8.3 °C to 13.8 °C in winter and from 20.2 °C to 25.8 °C in summer. Water temperature in winter was significantly different ($p < 0.05$) from that in summer. α-Tocopherol accumulation in pepper fruits differed significantly ($p < 0.05$) between winter (0.77 ± 0.13%) and summer (1.64 ± 0.21%). Changes in water temperature due to seasonality caused variations in α-tocopherol accumulation in pepper fruits, with fruits in the warmer season (summer) showing a higher accumulation of α-tocopherol. The warmer season is therefore a better period for α-tocopherol biosynthesis.

Significance:

This study evaluated whether variations in seasonal temperature in South Africa can affect α-tocopherol accumulation in pepper fruits grown in an aquaponic system. An aquaponic system could provide a sustainable solution for South Africa, with its diverse seasons and scarcity of fresh water. Determining the optimal temperature for increased α-tocopherol biosynthesis in higher plants improves our understanding of a favourable climate for a sustainable vitamin E source.

Introduction

Vitamin E is also known as tocochromanol. It is a vital micronutrient for humans.[1] The synthesis of vitamin E occurs in most photosynthetic plants and some algae.[1] There are four different forms of vitamin E (α, β, γ and δ).[2] Owing to its antioxidant activity, it can neutralise free radicals into less reactive compounds.[3] Improper amounts of this vitamin in humans can cause many conditions such as Alzheimer's and cardiovascular diseases.[4] It mostly occurs in an unesterified form.[5] In nature, α-tocopherol is the predominant and biologically active form of this vitamin.[5]

The two main environmental factors that can influence crop production are temperature and light intensity.[6] A temperature above or below the optimal can decrease the photosynthetic rate in higher plants, which influences the precursor pool by reducing the biosynthesis of secondary metabolites.[7] Higher heat stress induces the accumulation of tocopherols, especially α-tocopherol.[8] An optimal level of α-tocopherol is required to maintain the stability of chloroplasts and protect photosystems from singlet oxygen savaging.[8]

Studies concerning effects of seasonal changes in water temperature on α-tocopherol accumulation in pepper fruits are scarce. However, it has been reported that climatic changes are associated with increased carotenoid production in plantains.[9] According to another report, seasonal shifts affect the levels of total phenolics and flavonoids in vegetable plants.[10] In addition, low temperatures and high humidity have been linked to reduced levels of chlorophyll *a* and *b* and carotenoids in cucumber.[11] Therefore, this study was conducted to determine the influence of seasonal variations in water temperature on α-tocopherol accumulation in pepper fruits grown in an aquaponic system.

Materials and methods

Aquaponic site

The aquaponic greenhouse was located in Makhanda (formerly Grahamstown) in the Eastern Cape Province, South Africa. The system was exposed to only ambient sunlight and set up as a coupled commercial system.

Water temperature

The temperature of the fish (*Oreochromis mossambicus*) water, which had a stocking density of 30 kg in 1500 L, was measured directly from the tap using a portable dissolved oxygen meter (Lutron model PDO-520, Taiwan). The tap provided the nutrient water to the pepper growing on the gravel stone beds. Water temperature was measured twice weekly from 29 June 2020 to 31 August 2020 in winter. The water temperature measurements in the summer started from 3 December 2020 to 4 February 2021 in summer.

Plant material collection

Seventy matured red pepper (*Capsicum frutescens* L.) fruits were collected each season from four plants grown in two media beds after 8 weeks of water temperature measurements. The pepper fruits were collected on 31 August 2020 (winter) and 4 February 2021 (summer). In this single-trial study, Fe-EDDHA (500 g) and Sea-Brix (2.0 L) were given as supplementation in winter and summer, respectively.

Chemical reagents and apparatus

The standard (α-tocopherol) was sourced from Sigma-Aldrich, USA; high-performance liquid chromatography (HPLC)-grade ethanol was provided by Merck, Germany; ascorbic acid was obtained from Merck, South Africa; Whatman No. 1 filters were purchased from Whatman, England; and syringe filters were acquired from Goppingen, Germany.

Sample extraction

The pepper fruits were ground into a powder and 0.1% ascorbic acid was added to 5 g of the ground powder to reduce oxidation. Ethanol (30 mL) was then added and the sample was vortexed for 10 min before centrifugation at 1.750 x *g* for 10 min. The extract was filtered using a Whatman No. 1 filter. The filtrate was then evaporated to dryness using a rotary evaporator under reduced pressure. The residue (4.0 mg) was suspended in ethanol (2.0 mL) and filtered through a 0.22 μm syringe filter. Finally, the filtrate was injected into the HPLC for chromatography.

Chromatographic conditions

The HPLC consisted of a connector, a pump, an autosampler and a diode array detector. The detection wavelength ranged from 190 nm to 800 nm. Data acquisition and analysis were conducted with 'LC Lab Solution' software. A Luna® column (150 × 4.6 mm, 5 μm C18 (2) 100A) was used for the chromatographic separation. The mobile phase consisted of ethanol and water in a ratio of 97:3 v/v. The flow rate was 1.5 mL/min. The analyte injection volume was 12 μL for a 10 min run. The analyte was detected at a wavelength of 298 nm.

Statistical analysis

Data analysis was carried out on three replicates using a Student's t–test in Microsoft Excel 365® (Microsoft Corporation, New York, USA). The statistical level of significance was 5%.

Results and discussion

Figures 1 and 2 depict the winter and summer water temperatures, respectively. In winter, the minimum and maximum water temperatures were 8.3 °C and 13.8 °C, respectively (Figure 1). By comparison, the lowest and highest water temperatures in summer were 20.2 °C and 25.8 °C, respectively (Figure 2). Water temperature differed significantly ($p < 0.05$) between summer and winter.

The accumulation of α-tocopherol in the pepper fruits varied significantly ($p <$ 0.05) between winter (0.77 ± 0.13%) and summer (1.64 ± 0.21%) (Table 1). The summer results indicated greater accumulation of α-tocopherol; however, it was found to be lower in the winter (Table 1).

Plants are exposed to different abiotic factors that can influence their growth and productivity.[12] Low-temperature stress prevents the growth of plants, resulting in reduced metabolite production.[11] However, in higher plants, heat stress has been reported to increase the accumulation of secondary metabolites.[13] Microorganisms in aquaponic systems are able to mineralise organic compounds if the water temperature is between 25 °C and 30 °C.[14] Nutrient availability is optimal between 21 °C and 24 °C.[15] Hence, in this study, water temperatures of ±25 °C in the summer were relatively optimal.

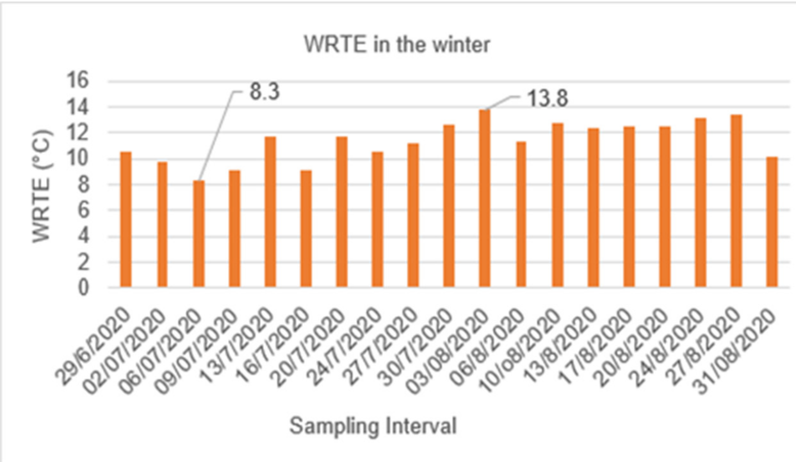

Figure 1: Water temperature (WRTE) measurements in winter. The highest and lowest water temperature values were 8.3 °C and 13.8 °C, respectively.

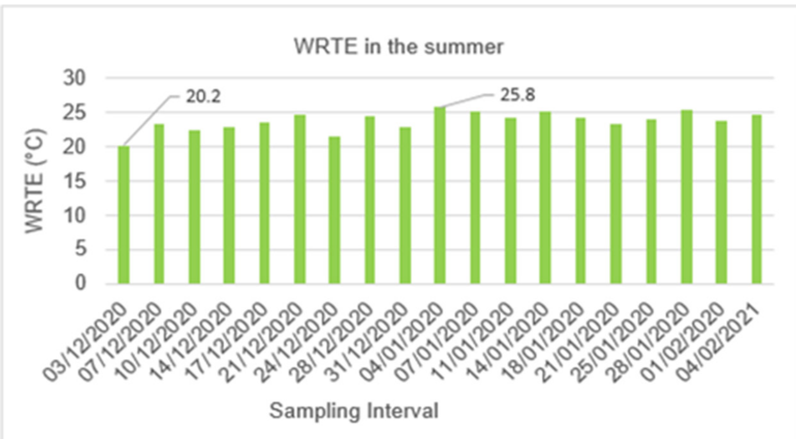

Figure 2: Water temperature (WRTE) measurements in summer. The highest and lowest water temperature values were 20.2 °C and 25.8 °C, respectively.

Table 1: Seasonal differences in α-tocopherol accumulation in the pepper fruits

Plant material	Pepper fruits, dry weight ($n = 3$)	
Seasons	**Winter**	**Summer**
α-tocopherol (%)	0.77 ± 0.13*	1.64 ± 0.21*

*$p < 0.05$

Although reports on the effects of seasonal changes in water temperature on α-tocopherol accumulation in pepper fruits are lacking, α-tocopherol has been reported in tomatoes and peppers.[16] Temperatures ranging from 12 °C to 32 °C have been reported to decrease the accumulation of lycopene and carotenoids in tomatoes.[17] Furthermore, Siberian ginseng grown at an ambient temperature of 24 °C presented a relatively high total phenolic content.[18] In this study, the water temperature measured during the summer increased the accumulation of α-tocopherol. The lower concentration obtained in winter could be the inability of the plant to withstand cold stress.

Conclusion

The accumulation of α-tocopherol in pepper fruits was greater in summer than in winter. Thus, in higher plants, accumulation of α-tocopherol can be advantageous for providing adaptive tolerance to high temperature stress. Additional studies are needed to elucidate the mechanism of the biosynthesis.

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Data availability

The data supporting the results of this study are available upon request to the corresponding author.

Declarations

I have no competing interests to declare. I have no AI or LLM use to declare. This paper is based on a PhD dissertation entitled 'Influence of seasonal dynamics on water quality, microbiome and plant nutritional makers of aquaponic systems'. Ethical approval was obtained from Rhodes University Animal Research Ethics Committee (approval numbers 2020-1221-3379 and 2020-2826-4868).

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