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*RESEARCH ARTICLE*

# EFFECT OF SEASONAL DYNAMICS IN ABIOTIC FACTORS ON β-CAROTENE BIOSYNTHESIS IN GREEN LEAFY VEGETABLES FROM AN AQUAPONIC GREENHOUSE

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## ABSTRACT

The present study was aimed to determine the effects of seasonal changes in pH, water temperature (WT), and electrical conductivity (EC) on β-carotene biosynthesis in green spinach (GRSP) and green lettuce (GRLE) leaves from an aquaponic greenhouse. The pH values of the fish water tap (FWTP) and deep-water culture tank (DWCT) were measured with a portable electrode pH meter. The WT and EC of the FWTP and DWCT were measured using a portable dissolved oxygen meter and multi-parameter water quality meter, respectively. The biosynthesis of β-carotene in the GRSP and GRLE was determined using HPLC-Shimadzu Prominence. This research was conducted over four consecutive seasons (winter, spring, autumn, and summer). The pH values of the FWTP and DWCT ranged from 6.39±0.33–7.43±0.43. The pH levels were significantly differed among some seasons. The maximum and minimum pH values were detected in the winter and autumn, respectively. The WT values of these components (FWTP and DWCT) ranged from 12.12±1.34–25.21±0.95 °C. The EC levels ranged from 0.55±0.05–0.65±0.55 mS cm-1. The WT and EC values differed significantly across four seasons. The highest and lowest WT and EC levels were observed in the summer and winter, respectively. The biosynthesis of β-carotene in the GRSP and GRLE leaves ranged from 3.63±0.27–25.80±6.67%. A significant difference in β-carotene biosynthesis was detected among some periods. The highest level of β-carotene biosynthesis in both leafy vegetables occurred in the summer. The results of this study revealed that the seasonal dynamics of pH, WT, and EC induced variations in β-carotene biosynthesis in the studied leafy plants. Therefore, the results of this work could be used to understand better climate and growing conditions for sustainable food production.

**KEYWORDS**

Green spinach, portable dissolved oxygen meter, sustainable food production, summer, water temperature

## 1. INTRODUCTION

One of the major environmental factors influencing secondary metabolite metabolism in plants is temperature fluctuations (Delker et al., 2014; Quint et al., 2016). A warmer or colder temperature above or below the optimum range can reduce the photosynthetic rate of plants (Paredes and Quiles, 2015; Drake et al., 2018). This in turn decreases the metabolite precursor pool for the biosynthesis of secondary metabolites such as carotenoids. Stress due to heat can cause chloroplasts to swell, inhibiting photosynthesis, and thus plastoglobulin formation (Zhang et al., 2010). The amount of carotenoid biosynthesis in plants is species and tissue dependent in response to ambient temperature. A temperature of 30 °C is optimal for β-carotene biosynthesis in Spirulina platensis microalga (Hamidi et al., 2023). Ambient temperatures above the range of 12 to 32 °C decrease the biosynthesis of lycopene and other carotenoids (Dumas et al., 2003; Hernandez et al., 2015). Another report revealed that an ambient temperature of 35 °C can downregulate carotenoid biosynthesis in plants (Tran and Raymundo, 1999).

A low pH value (5.0) is suitable for the optimal enzymatic production of β-carotene in grape juice medium by Saccharomyces. cerevisiae (Luo et al., 2013). Also, a pH greater than or equal to 7.0 yielded optimal β-carotene biosynthesis in Spirulina platensis (Hamidi et al., 2023). In addition, at relatively high pH (9.0), Dunaliella bardawil results in increased production of β-carotene (Khalil et al., 2010). Therefore, the pH of an environment could determine β-carotene production.

Nutrients play crucial roles in chloroplast development and carotenoid biosynthesis in plants (Stitt and Krapp, 1999; Jin et al., 2015). Hence, an excess or deficiency in nutrients can affect carotenoid production in plants (Myśliwa-Kurdziel and Strzałka, 2002; Dahmen-Ben Moussa et al., 2017). Additionally, insufficient levels of a particular metal ion(s) in the soil or water can disrupt chloroplast development and carotenoid synthesis in plants (Myśliwa-Kurdziel and Strzałka, 2002; Baek et al., 2012). For example, iron deficiency impairs chloroplast thylakoid membranes, causing chlorosis in plants (Lopez-Millan et al., 2013). The importance of Fe3+ in carotenoid biosynthesis is highlighted by its role in the mediation of redox regulation during zeta-carotene isomerization (Beltran et al., 2015). Furthermore, nitrogen availability can increase chloroplast development and carotenogenesis in plants (Cetner et al., 2017). For example, nitrogen availability has been shown to increase β-carotene accumulation in vegetables such as carrots spinach and brassica (Reif et al., 2013; Boskovic-Rakocevic et al., 2012; Lefsrud et al., 2007). Conversely, nitrogen deficiency or starvation can lead to the breakdown of chloroplast thylakoid membranes and photosynthetic pigments, causing yellowing of plant leaves (Cetner et al., 2017). Photosynthetic pigments, such as carotenoids, trap photons of solar radiation to initiate electron transport, converting light energy into chemical energy in the leaves of

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photosynthetic plants (Qin et al., 2015; Nolan, 2018).

Carotenoids are 40-carbon lipophilic dietary nutrients that are vital for good visual acuity and detoxification of reactive oxygen species (Xavier and Perez-Galvez, 2016; Nolan, 2018). β-carotene is a type of carotenoid, main precursor of vitamin A synthesis in humans (Guevara-González et al., 2009). Additionally, β-carotene is an essential metabolite in high demand as a natural coloring agent in the food and cosmetic industries (Guevara-González et al., 2009). The biosynthesis of β-carotene in plants can increase under greenhouse conditions (Guevara-González et al., 2009). Greenhouse structures can protect plants from adverse environmental factors such as wind, rain, pests, and insects, which increase nutrient composition (Guevara-González et al., 2009).

Spinach (Spinacia oleracea) is native to central and southwestern Asia and grows well in aquaponic greenhouses (Shete et al., 2013; Goddek et al., 2015; Hu et al., 2015). This green leafy vegetable requires high moisture, making it perfect for the system. It is edible and belongs to the genus and family Amaranthus and Amaranthaceae, respectively (Shete et al., 2013; Goddek et al., 2015; Hu et al., 2015). Spinach grows to a height of 30 cm with variable leaf shapes that range from alternate, simple, ovate, and triangular (Sani et al., 2011). It can survive best within the temperature range of 4.4–15 °C and pH range of 6.4–6.8 (Sani et al., 2011). There are three main types of spinach: savoy, semisavoy, and smooth leaf (Ware and Olsen, 2023). Chlorophylls a and b are the major photosynthetic pigments of spinach. Carotenoids (β-carotene, lutein) are other pigments present in minor quantities. These pigments are found in the thylakoid membranes of chloroplasts and capture light energy during photosynthesis (Han et al., 2023). The level of these pigments can be affected by exposure to light, temperature, storage conditions, and nitrate availability spinach is a nutritious vegetable that possessed numerous health benefits. It is rich in vitamins, minerals, and antioxidants (Han et al., 2023). It contains high levels of iron, which is crucial for healthy blood to support oxygen transport in the body (Assimakopoulou, 2006). It is loaded with vitamin A, vitamin C, and vitamin K, which increase immunity, improve skin health, and strengthen bone, respectively (Butt and Sultan, 2018). Lutein and zeaxanthin are antioxidants found in spinach, and are essential for healthy eyes. Owing to its anti-inflammatory properties, spinach can reduce inflammation in arteries, thereby lowering the risk of cardiovascular diseases (Tang et al., 2017). The fiber content of this vegetable plant promotes gut health and regulates blood glucose levels (Roberts and Moreau, 2016). Magnesium in spinach is vital for energy production and muscle support (Chaudhari et al., 2024).

Lettuce (Lactuca sativa L.) was originally farmed by ancient Egyptians and belongs to the Asteraceae family (Simeonidou et al., 2012). It is popularly used in salads ((Simeonidou et al., 2012; Buzby and Lin, 2014; Wahyuningsih, 2015) and is widely cultivated and consumed as a leafy plant globally, with China being the largest producer (Mou, 2012). Lettuce grows faster in aquaponic greenhouses than in soil (Resh, 2001; Sace and Fitzsimmons, 2013). It is one of the first leafy vegetables cultivated commercially in hydroponic systems (Resh, 2001). The faster growth cycle allows for the harvest of lettuce within four to five weeks, leading to quick profits and nutrient turnover (Castro et al., 2006). There are numerous types of lettuce, but on the basis of leaf shape, texture, size, stem type, and head formation, there are six main types: crisp-head, butter-head, romaine, cut stalk, asparagus, and Latin lettuce (Mou, 2012). The main photosynthetic pigments in lettuce leaves are chlorophylls a and b, carotenoids, and anthocyanins. The primary green pigments are the chlorophylls, critical for photosynthesis; and carotenoids, responsible for the orange and yellow color. Anthocyanins contribute to the red and purple color. The accumulation of these colors depends on environmental conditions such as the temperature and intensity of light (Li et al., 2021). Lettuce is rich in moisture (94–95%) (Yang et al., 2022). It is a rich source of vitamins, mineral elements, and secondary metabolites such as polyphenols (Yang et al., 2022), vitamin C (Medina-Lozano et al., 2021), and carotenoids (Mitra et al., 2021). Thus, lettuce can prevent diabetes, oxidative damage, some cancers, and Alzheimer's disease (Naseem and Ismail, 2022; Kabir et al., 2021).

An aquaponic greenhouse is a sustainable farming practice for the production of fish and vegetable plants, offering a potential solution to the major problems associated with food production to feed the growing world population (Castro et al., 2006; Diver, 2006,). The greenhouse enables the production of more plants per square foot than traditional agricultural methods because of close proximity of planting (Rakocy et al., 1993. Additionally, plant roots absorb the required nutrients without competition from nonindigenous plants such as weeds, unlike conventional practices that rely on fertilizer application (Savidov, 2004; Savidov and Hutchings, 2005). Furthermore, an aquaponic is typically set up inside greenhouse to minimize the adverse effects of biotic and abiotic factors, leading to increased growth, yields, and nutritional compositions (Blidariu and Grozea, 2011; Brook, 2022). Edible plants grown in aquaponic greenhouses are categorized into fruits (such as tomatoes, eggplants, peppers, and chilies), leafy plants (such as lettuce, mint, and spinach), flowers (such as broccoli), bulbs (including garlic and onion), and roots (such as carrots) (Ukom and Obi, 2018). These edible plants contain valuable metabolites that can benefit human health and nutrition including tocopherols, carotenoids, saponins, alkaloids, tannins, polyphenolics, and flavonoids (WHO, 2003; Kris-Etherton et al., 2021; Karasawa and Mohan 2018; Braglia et al., 2021).

Reports on the effects of seasonal variations in pH, water temperature, and nutrient availability on β-carotene biosynthesis in plants grown in aquaponic greenhouses are scarce. The analysis reported that changes in climate increased the levels of carotenoids in certain plantain cultivars (Dzomeku et al., 2020). Study reported the influence of seasonal dynamics on chlorophyll and carotenoids in the leafy plants from steppe and forest environments (Ivanova et al., 2020). Another report suggested that changes in climate can affect the productivity and composition of plants (Herrera et al., 2017). Therefore, this study was intended to investigate how seasonal changes in pH, water temperature, and electrical conductivity affect β-carotene biosynthesis in green spinach and green lettuce leaves grown in an aquaponic greenhouse.

## 2. MATERIALS AND METHODS

### 2.1 Study site and set–up

The research study was carried out in an aquaponic greenhouse located in the town of Makhanda (Grahams town), Eastern Cape, South Africa. It was set up as a coupled commercial greenhouse exposed to only ambient sunlight. The greenhouse consists of fish tanks (4 × 1,500 L), sump tanks (1 × 1,500 L and 1 × 500 L), flood-and-drain gravel stone media beds (20 × 400 L), and deep-water culture tanks (24 × 900 L). The fish, deep-water culture, and sump tanks have associated submersible pumps (SOBO®, WP-7000, 105 W, 5000 L H-1). The system components were connected with PVC pipes to form a closed loop.

### 2.2 Water sample collection

Each water sample from the fish water tap (FWTP) and deep-water culture tank (DWCT) was collected into a clean 50 mL screw-capped Falcon tube, placed on ice, and transported immediately to the laboratory for electrical conductivity (EC) measurement. Water temperature (WT) and pH measurements were carried out directly from each of the FWTP and DWCT components in the aquaponic greenhouse. The pH, WT, and EC analyses in the winter were conducted from June 29, 2020, to August 31, 2020. The spring measurement started on September 3, 2020, and continued until November 30, 2020. In the summer, the determination commenced on December 3, 2020, and lasted until March 1, 2021. Finally, the autumn evaluation began on March 4, 2021, and ended on May 27, 2021. The analysis of each water sample was carried out twice weekly (Mondays and Thursdays) throughout the study period.

### 2.3 Plant sample collection

Green spinach (GRSP) leaves (silver-beet cultivar) were harvested from a gravel stone media bed (GSMB). Green lettuce (GRLE) leaves (Locarno cultivar) were obtained from a polystyrene sheet in deep-water culture tank (DWCT). The fish water tap (FWTP) and deep-water culture tank (DWCT) supplied nutrient water to the GRSP and GRLE plants. Figure 1 displays the study vegetable plants. The leaf sample of each plant was collected on August 21, 2020, in the winter. On November 24, 2020, was for the spring. On March 1, 2021, was for the summer. Finally, on May 30, 2021, was for the autumn. Each sample was placed in a separate clean plastic bag and transported to the laboratory. Upon arrival, each plant material was rinsed with Milli-Q water to remove contaminants. Each sample was separately air-dried in an oven at 30 °C, ground into powder, and stored in screw-capped Falcon tubes.

**Figure 1**: Study leafy plants. (a) Green spinach (GRSP) of the Silver-Beet cultivar in a wicking bucket to support the growing roots and (b) Green lettuce (GRLE) of the Locarno cultivar. The GRSP was grown on a gravel

stone media bed. The GRLE was cultivated on polystyrene rafts in deep-water culture tank.

### 2.4 Reagents and apparatus

The reagents and apparatus used were HPLC-grade β-carotene (Sigma–Aldrich, St. Louis, USA), HPLC-grade tetrahydrofuran (Merck, EMD Millipore Corporation, Germany), a Minisart syringe filter (0.22 µm) (Goppingen, Germany, LOT 00807103), Milli-Q water (EMD-Millipore machine, Switzerland), and HPLC amber vials. The equipment used consisted of HPLC-Shimadzu-UFLC Prominence system (Shimadzu Corporation, Kyoto, Japan), Luna®, 5 µm C18 (2) 100A column (150 × 4.6 mm) (Phenomenex, USA), a portable dissolved oxygen meter (Model: PDO-520, Taiwan), a multi-parameter water quality meter (PHT-27, China), a portable electrode pH meter (Model: PH-220, Taiwan), an analytical weighing balance (RADWAG, 220 g × 0.1 mg, Model, AS/220/C/2, Poland), a BÜchi heating water bath (B-491, Switzerland), and a BÜchi rotary evaporator (R-210, Switzerland). All the reagents and equipment used in this study were of analytical grade.

### 2.5 Standard preparatio

The stock solution of β-carotene (HPLC-grade) was prepared following the methods reported by Gleize et al. (2012) and Yokoto and Oshio (2017) with modifications in concentration values. To prepare a 2.0 mg/mL stock solution, 10 mg of β-carotene was dissolved in 5.0 mL of tetrahydrofuran (HPLC grade). A working solution of β-carotene (1,000 µg mL-1) was generated from its stock. Various concentrations of 2.5, 5, 10, 15, 40, 100, 200, 400, 500, and 1,000 µg mL-1 were prepared from the working solution by further dilution with tetrahydrofuran. Prior to injection into the HPLC-machine, all working solutions were filtered through a 0.22 µm acro-disc syringe filter.

### 2.6 Sample extraction

Each dried sample (5.0 g) of green spinach and green lettuce was placed in separate Falcon tubes with the addition of 2.0 mL of ascorbic acid (0.1%) to prevent oxidation. Methanol/dichloromethane (dilution solution, 2:1 v/v) (30 mL) was added, and the mixture was vortexed well for 15 min. Each mixture was then centrifuged at 4,000 rpm for 20 min. The supernatant of each mixture was then filtered through Whatman filter paper (11 µm pore size) and evaporated to dryness with a rotary evaporator under reduced pressure at 25 °C. Each evaporated sample residue (2.0 mg) was suspended in 1.0 mL of the dilution solution and filtered through a 0.22 µm Minisart syringe filter before injection into the HPLC system for chromatography. The remaining residues were preserved at -20 °C. Sample extraction was performed at room temperature (25±5 °C) in the dark to minimize possible light-induced isomerization. The sample extraction method is analogous to that of Gleize et al. (2012).

### 2.7 Analytical methods

#### *2.7.1 pH, water temperature and electrical conductivity assessment*

The pH was measured with a portable electrode pH meter (Model: PH-220, Taiwan), the water temperature (WT) was assessed using a portable dissolved oxygen meter, and the electrical conductivity (EC) of the water was determined using a multi-parameter water quality meter.

#### *2.7.2 β-carotene analysis*

Each standard solution and sample extract was injected in triplicate into the UV-HPLC-Shimadzu-UFLC Prominence system with LC-20AD connector, LC-2AB pump (20 MPa), SIL-2A auto sampler, and SPDA-M20A diode array detector. The diode array detector (DAD) wavelength was set between 190 and 800 nm. The "LC Lab Solution" software was used for HPLC data acquisition and analysis. Chromatographic separation was performed with a Luna®, 5 µm C18 (2) 100A column (150 × 4.6 mm). The mobile phase delivery was isocratic and consisted of tetrahydrofuran/water at a ratio of 97:3, v/v. The flow rate was 1.0 mL/min. The column was maintained at room temperature (25±5 °C). The injection volume was 10 µL for 10 min run. The ultraviolet-visible (UV-vis) absorbance was detected at a wavelength of 450 nm.

### 2.8 Statistical analysis

The study data were statistically analyzed through repeated-measures analysis of variance (RM ANOVA) using Microsoft Excel 365® (Microsoft Corporation, New York, USA). A significance level of 5% was used. If RM ANOVA indicated a significant difference among the four comparative seasons, a post hoc test (unpaired Student's t-test) was subsequently conducted to identify the specific point(s) where a significant difference existed.

## 3. RESULTS AND DISCUSSION

The pH levels of the fish water tap (FWTP) and deep-water culture tank (DWCT) significantly differed in the winter compared with the summer (p-value < 0.02) and the winter in compared with the autumn (p-value < 0.03). Similarly, the spring differed significantly from summer (p-value < 0.04) and the spring compared with the autumn (p-value < 0.04) (Table 1). Whereas, no significant difference was detected between winter and spring (p-value < 0.10) (Table 1). The water temperature (WT) values of the FWTP and DWCT components significantly differed between winter and spring (p-value < 0.003), between winter and summer (p-value < 0.0001), between winter and autumn (p-value < 0.004), between spring and summer (p-value < 0.003), between spring and autumn (p-value < 0.02), and between summer and autumn (p-value < 0.003) (Table 1). Likewise, the electrical conductivity (EC) levels of these two components (FWTP and DWCT) differed significantly between winter and spring (p-value < 0.005), winter and summer (p-value < 0.0003), winter and autumn (p-value < 0.0007), spring and summer (p-value < 0.001), spring and autumn (p-value < 0. 004), as well as summer and autumn (p-value < 0. 005) (Table 1). Both the FWTP and DWCT components revealed similar trends in pH, WT, and EC levels (Table 1).

Table 1: pH, water temperature (WT) and electrical conductivity (EC) levels of the fish water tap (FWTP) and deep-water culture tank (DWCT) during the four seasons

**Table 1:** pH, water temperature (WT) and electrical conductivity (EC) levels of the fish water tap (FWTP) and deep-water culture tank (DWCT) during the four seasons

| Water Quality Parameters | Experimental Components | Winter (June 2020 to August 2020) (n = 22) | Spring (September 2020 to November 2020) (n = 24) | Summer (December 2020 to March 2021) (n = 24) | Autumn (March 2021 to May 2021) (n =24) |
|---|---|---|---|---|---|
| pH | FWTP | $7.43{\pm}0.43^{a}$ | $7.27{\pm}0.73^{a}$ | $6.39{\pm}0.33^{b}$ | $6.73{\pm}1.38^{b}$ |
| | DWCT | $7.42{\pm}0.99^{a}$ | $7.30{\pm}0.48^{a}$ | $6.48{\pm}0.33^{b}$ | $6.72{\pm}1.22^{b}$ |
| WT (˚C) | FWTP | $12.20{\pm}1.42^{a}$ | $18.05{\pm}2.44^{b}$ | $25.21{\pm}0.95^{c}$ | $19.74{\pm}1.91^{d}$ |
| | DWCT | $12.12{\pm}1.34^{a}$ | $18.10{\pm}2.95^{b}$ | $24.93{\pm}0.91^{c}$ | $19.97{\pm}3.07^{d}$ |
| EC ($mS\ cm^{-1}$) | FWTP | $0.56{\pm}0.05^{a}$ | $0.59{\pm}0.04^{b}$ | $0.64{\pm}0.04^{c}$ | $0.62^{c}{\pm}0.05^{d}$ |
| | DWCT | $0.55{\pm}0.05^{a}$ | $0.60{\pm}0.04^{b}$ | $0.65{\pm}0.05^{c}$ | $0.61^{c}{\pm}0.05^{d}$ |

Each abiotic factor was measured twice a week (Mondays and Thursdays) for the entire research period. The results are presented as the means ± SDs. Values with different superscript letters between seasons differ significantly. WT = water temperature, EC = electrical conductivity, FWTP = fish water tap, DWCT = deep-water culture tank, n = number of times the water sample was collected per component per season.

Figure 2: illustrates the seasonal changes in pH, water temperature (WT), and electrical conductivity (EC) throughout the four comparative seasons (94 days). The highest and lowest pH values were obtained in the winter and autumn, respectively. However, the maximum and minimum WT levels were recorded in summer and winter, respectively. Similarly, the

highest and lowest EC values were detected in the summer and winter, respectively.

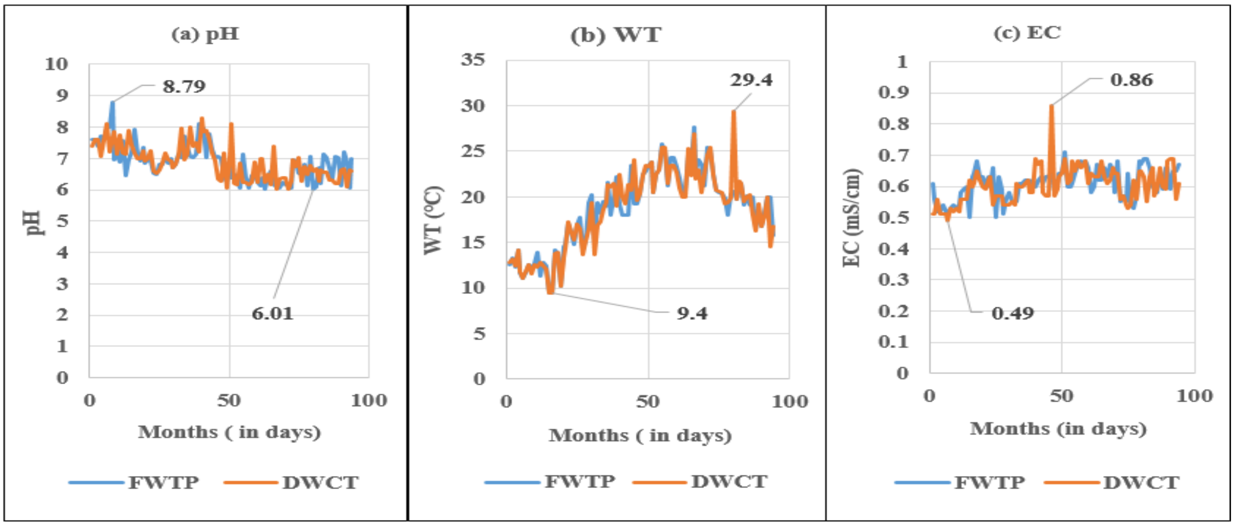

**Figure 2:** Seasonal variation in the (a) pH (b) water temperature (WT) and (c) electrical conductivity (EC) values for the entire study period (94 days). The highest (8.79) and lowest (6.01) pH values were obtained in the winter and autumn, respectively. The maximum (29.4 °C) and minimum (9.4 °C) WT levels were detected in the summer and winter, respectively. Similarly, the highest (0.86 mS cm-1) and lowest (0.49 mS cm-1) EC values were recorded in the summer and winter, respectively. DWCT = deep-water culture tank, FWTP = fish water tap.

β-carotene biosynthesis in green spinach (GRSP) leaf was highly significant in the summer (25.80±6.67%) and autumn (17.19±2.08%) (p-value < 0.001) (Figure 3). Similarly, the biosynthesis of β-carotene in green lettuce (GRLE) leaves differed significantly between summer (15.36±5.95%) and autumn (10.11±5.06%) (p-value < 0.01) (Figure 3). However, β-carotene biosynthesis in GRSP leaves did not differ significantly between winter (10.57±0.21%) and spring (9.36±3.37%) (p-value > 0.09) (Figure 3). Similarly, the biosynthesis of this compound (β-carotene) in GRLE leaves differed significantly between winter (4.13±4.55%) and spring (3.63±287%) (p-value > 0.17) (Figure 3). The GRSP leaves presented the highest β-carotene biosynthesis in the summer whereas, the GRLE leaves presented the lowest in the spring period (Figure 3).

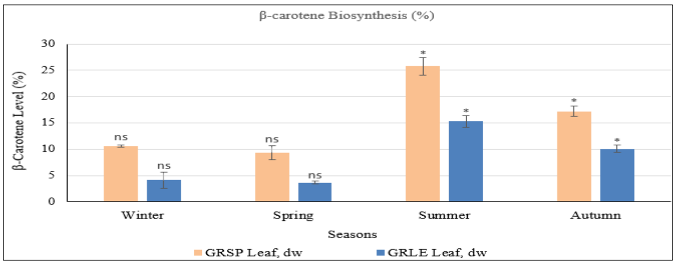

**Figure 3**: Seasonal differences in β-carotene biosynthesis among the four seasons for the two green leafy materials. GRSP = green spinach, GRLE = green lettuce, dw = dry weight, ns = no significant difference, * = significant variation.

Aquaponic greenhouse technology is a crucial tool for increasing the quantity and quality of vegetable food production. The dark green color of leafy spinach and lettuce indicates high levels of health-promoting carotenoids and chlorophylls (Petrea et al., 2013). β-carotene is an active form of carotenoid known as provitamin A (Scott and Rodriquez-Amaya, 2000; Ishida and Bartley, 2005). Numerous epidemiological studies have demonstrated a strong relationship between β-carotene intake and a reduced risk of carcinogenesis, cardiovascular disease, neuronal damage, inflammation, and macular degeneration (Cantuti-Castelvetri et al., 2000; Yamaguchi and Uchiyama, 2003). An adult's normal blood level of β-carotene is approximately 9.0 µg mL-1 (Beers, 2006; USDA, 2019).

This study investigated the effects of seasonal variations in pH, water temperature (WT), and electrical conductivity (EC) on β-carotene biosynthesis in green spinach (GRSP) and green lettuce (GRLE) leaves from an aquaponic greenhouse. According to Tyson et al. (2011), when the pH value is below 4.0 or above 8.0, the process of nitrification and nutrient levels decreases. For the optimal nitrification process and nutrient availability to plants, a pH ranging from 6.5–7.0 is adequate (Somerville et al., 2014).

Most plants require an optimal WT between 21 and 24 °C to thrive and grow properly (Sallenave, 2016). However, some local varieties can survive well outside the above range (Sallenave, 2016). The ability of microbes in the aquaponic system to metabolize and convert organic or inorganic compounds into plant nutrients is enhanced if the water temperature is optimal (25–30 °C) (Sallenave, 2016). A WT below the optimal value can affect the availability of nutrients to plants (Helene and Ivar, 2020).

The EC is a measure of the total amount of dissolved ions or nutrients in water (Manju et al., 2017; Nagayo et al., 2017). Dissolved ions such as nitrate, phosphate, potassium, zinc, and chloride can increase the electrical conductivity of water, which in turn affects the nutrients available to plants (Brinkop and Piedrahita, 1996). The recommended level of EC in aquaponic systems ranges from 0.3–0.6 mS cm-1 (Rakocy et al., 2006). In this study, the pH and WT in the summer were approximately 6.5 and 25 °C, respectively. Therefore, the observed optimal values of EC during this period could be attributed to the increased microbial decomposition of organic matter (such as fish feces and feed remains) and nitrification processes.

The presence of β-carotene in spinach and lettuce leaves was reported as detected in the present study (Isma'il and Fun, 2003; Ahmad et al., 2007; Ju et al., 2021). During the summer, greater amounts of β-carotene in GRSP and GRLE leaves could be attributed to increased biosynthesis, which can be linked to optimal levels of pH (±6.5), WT (±25 °C), and EC (±0.63 mS cm-1) during values the season (summer).

Interestingly, the findings of this study are in line with those of (Bell et al., 2016). They reported that a relatively acidic pH of 6.0 increased the total carotenoid content in carrot juice, but slightly basic (8.0) or neutral (7.0) pH values decreased the total carotenoid content. The study reported that total carotenoid production is optimal in Euglena sp. when the pH (4.5) is acidic (Nurafifah et al., 2023). On the other hand, the findings of the present study did not support those of Khalil et al. (Khalil et al., 2010). They reported that relatively high pH (9.0) promoted increased production of ▯-carotene in Dunaliella bardawil.

In addition, the results of this research support the findings of (De Azevedo-Meleiro and Rodriguez-Amaya, 2005). They reported that the carotenoid contents in New Zealand and endive spinach were relatively high in the summer. The analysis reported that increased UV radiation increases carotenoid accumulation in tobacco leaves (Shen et al., 2017). In contrast, reported that the exposure of tomatoes to temperatures above 12–32 °C decreases lycopene and carotenoid biosynthesis (Dumas et al., 2003; Hernandez et al., 2015). Study reported that a temperature of 35 °C decreases carotenoid biosynthesis in bitter melon (Tran and Raymundo, 1999).

Analysis reported that a relatively high potassium (K) level in a nutrient solution increases the production of ▯-carotene in tomatoes (Martin-Hernandez et al., 2022). To study reported that carotenoid biosynthesis in carrots decreased with increased in nitrogen (N) concentration (Oleszkiewicz et al., 2025). Therefore, in this study, the slightly acidic pH and relative increases in WT and EC due to seasonal changes induced increases in β-carotene biosynthesis in the two green leafy materials.

## 4. CONCLUSION

β-carotene accumulates in relatively high amounts in the leaves of the studied plant species during the summer, which is the warmest season. This accumulation can provide an additional adaptive advantage of relatively high temperature stress tolerance in higher plants. As a food-based strategy, the studied plant materials could be good sources of sustainable vitamin A, especially in developing countries. However, the bioavailability, conversion, and absorption of β-carotene in the body could be challenging. Further studies are needed to better understand the mechanisms of -carotene biosynthesis and its functions as well as other forms of carotenoids in the studied green leafy vegetables and related species.

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## DECLARATION OF INTEREST

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