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Research article

Effect of Pre-harvest Periods by Replacing Nutrient Solution with Tap Water on Nitrate and Quality in Hydroponic Lettuce

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Abstract: Lettuce (*Lactuca sativa* L.) is a high-value nutritional food for human consumption, rich in minerals and vitamins. However, people are still concerned about the residues of nitrate content in hydroponic lettuce. Pre-harvesting by replacing nutrient solution with tap water is one of several factors that can help to reduce nitrate content and improve the quality of hydroponic lettuce. Therefore, this study focused on the effect of pre-harvest periods by replacing nutrient solution with tap water on nitrate and quality in hydroponic lettuce. This experiment was performed by replacing nutrient solution with tap water in a hydroponic system for 0 h, 24 h, 48 h, and 72 h before harvesting the lettuce product. Nitrate, vitamin C, phenolic, and soluble sugar contents were determined. The results showed that nitrate content was lowest under pre-harvest conditions for 72 h (19 mg/g dry weight, 63% reduction), but it was not significantly different for 48 h (20 mg/g dry weight, 61% reduction). The vitamin C content was significantly decreased after pre-harvesting for 72 h (5.3 μg/100 g, 30% reduction). The soluble sugar content was significantly increased and reached its highest content after pre-harvest for 48 h (3.3 mg/100 g, 105% increase), but there was no significant difference at 72 h (3.2 mg/100 g, 98% increase). Although the phenolic content decreased slightly, there was no significant difference among the treatments. In conclusion, nitrate contents were reduced the lowest, and soluble sugar contents were increased the highest after pre-harvest for 48 h. Vitamin C and phenolic contents were slightly decreased after pre-harvest periods by replacing the nutrient solution with tap water.

Keywords: Green Oak Lettuce; Nitrate; Phytochemicals; Hydroponic System; Water Flow; Pre-harvest

1. Introduction

A hydroponic system is a suitable farming technology to produce fresh leafy vegetables rapidly, and it also has the potential to reduce the environmental impact of food production worldwide. A hydroponic farm has an efficient water and fertilizer system, pays lower labor costs, and promotes plant growth. Furthermore, vegetables grown in a hydroponic system are less likely to be contaminated with *Escherichia coli* and *Salmonella* spp [1]. Therefore, customers feel comfortable consuming raw vegetable products from a hydroponic farm as it offers sustainable, healthy food production and maintains a clean environment without heavy chemicals.

Lettuce (*Lactuca sativa* L.) has a high nutritional value for human consumption. Lettuce is rich in minerals and vitamins. Aćamović-Djoković et al. [2] reported that the vitamin C content of lettuce ranges from 3.50 to 9.60 mg/100 g of fresh weight. However, lettuce is not always good for consumer health, and several people are concerned about excessive nitrate content or nitrate residue, especially in hydroponic lettuce production. Hydroponic vegetables have the potential to accumulate high nitrate concentrations that are a risk to human health as they can produce carcinogenic N-nitroso compounds that cause gastrointestinal cancer [3-4]. The European Commission Regulation (No. 194/97, 1997) [5] suggests that the maximum nitrate concentration should not exceed 3.65 mg/kg of body weight daily. For example, 60 kg of body weight should not consume more than 219 mg per day.

Nitrate is one of the nitrogen sources that plays an essential role as a plant nutrient for the main constituents of chlorophyll, protein, and genetic material [6]. Plants take nitrate via the root hair, xylem, mesophyll cells, and cell walls. Then nitrate is stored in the cell and reduced into nitrite, ammonium, glutamine, and other amino acids [7]. After assimilating nitrate into glutamine acid, it is safe for human health. However, during the transportation of nitrate from the root to the leaf cells, nitrate is specially retained in the xylem vessels of the root, stem, petiole, and leaf veins. This nitrate molecule is still harmful to human health [8].

Pre-harvest is a practical method that can help to improve the quality and shelf-life of vegetables and reduce their risk before harvest [9–11]. For example, Gil [9] studied pre-harvest related to agricultural practices, including type and rate of fertilization, irrigation, and salinity, which affect fresh-cut quality, and found that pre-harvest factors increased the quality of fresh-cut lettuce and baby leaves. Arturas et al. [12] reported that pre-harvest with supplemental solid-state lighting on lettuce cultivars can improve the antioxidant properties of lettuce. Wanlai et al. [13] also studied the effect of pre-harvest illumination with a red and blue light ratio. They found that nitrate content was reduced and soluble sugar content was dramatically increased in lettuce under pre-harvest continuous illumination. Guffanti et al. [14] have reported that nitrate content has reduced and antioxidant activity has increased after exposure to light and replacing the nutrient solution with tap water before harvest in a vertical farm. Moreover, several research studies related light pre-harvest in lettuce [15–17]. However, there is less knowledge of pre-harvest periods by replacing nutrient solution with tap water, which can reduce nitrate and other synthetic fertilizers in the xylem of plants by replacing water in a hydroponic system. Therefore, this study focused on the effect of pre-harvest periods by replacing nutrient solution with tap water on nitrate and quality in hydroponic lettuce.

2. Materials and Methods

2.1 Plant materials and growth conditions

The green oak lettuce (*Lactuca sativa* L.) was used as plant material, and a nutrient film technique (NFT) hydroponic system was applied for cultivation. The seeds were sown on the wet tissue paper in the box and left at room temperature for three days. Then the seeds with a radicle of about 10 mm were transplanted into polyfoam cubes (2.5x2.5x2.5 cm), and placed in the nursery greenhouse for 2 weeks. Afterward, the seedlings were transplanted into the NFT system for this experiment. All the plants were grown under a 50% aluminum net shade with a temperature of 24 to 32 °C and a relative humidity of 62% to 92%. Modified Hoagland and Arnon [18] nutrient solution was used as a hydroponic nutrient solution with an electrical conductivity (EC) of 1.8 mS/cm and pH 5.5- 6.5 were adjusted once a week. Briefly, the nutrient stock composition was prepared as follows: In solution stock A, it contained; potassium nitrate (KNO_3, 780 g/10 L), mono ammonium phosphate ($NH_4H_2PO_4$, 130 g/10 L), magnesium sulfate ($MgSO_4{\cdot}7H_2O$, 500 g/10 L), manganese EDTA (MnEDTA, 8 g/10 L), monopotassium phosphate (KH_2PO_4, 100 g/10 L), and microelements (Boron EDTA, MnEDTA, MgO, CuEDTA, MoEDTA, and FeEDTA, 10 g/10 L). The stock B solution was comprised of calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$, 1000 g/10 L) and iron chelate (FeEDTA, 10 g/10 L). This experiment was performed at the Department of Agricultural and Fisheries Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus (latitude 6.882424 and longitude 101.235334).

2.2 Treatment and experimental design

This study focused on the effect of pre-harvest periods by replacing nutrient solution with tap water on nitrate and quality in hydroponic lettuce. Thus, lettuce plants experimented with 30 days after transplanting into the NFT system. The experiment was comprised of four treatments with different pre-harvest periods by replacing nutrient solution with tap water, including (1) 0 h (harvested before replacing nutrient solution with tap water, control treatment), (2) 24 h (harvested after replacing nutrient solution with tap water for 24 h), 48 h (harvested after replacing nutrient solution with tap water for 48 h), and 72 h (harvested after replacing nutrient solution with tap water for 72 h). A completely randomized design (CRD) with three replications was used in this experiment.

2.3 Determination of nitrate content

The samples of lettuce leaves were collected, washed with running tap water, and washed with distilled water three times. After that, the samples were dried at 65 °C for 24 h and then finely grounded. Next, 1 g of the samples were weighed, suspended in 10 mL of distilled water, stood at 45 °C for 1 h, and filtered through No. 40 Whatman filter paper. A 50 mL tube with 0.4 mL of 5% (w/v) salicylic acid was used, and 0.1 mL of the extraction was added. After that, the samples were left at room temperature for 20 min. 9.5 mL of a 2N sodium hydroxide (NaOH) solution was added to the samples. The absorbance was immediately applied at 412 nm (Biochrom, Libra S12, England) for determination [19].

2.4 Determination of soluble sugar content

The determination of soluble sugar content was done by Yemn and Willis method [20]. Lettuce leaves were dried at 65 °C for 48 h before being ground thoroughly. 50 mg of the dry sample was mixed in 4 mL of 80% ethanol, homogenized, transferred into test tubes, and then boiled in a water bath for 15 min. After that, the samples were centrifuged at 3,000 rpm for 15 min. The supernatant was removed. 2 mL of 80 % ethanol was added and recentrifuged. This step was repeated two times. The supernatant was collected and the final volume was 10 mL. After that, 0.2 mL of the aliquot was pipetted into test tubes and evaporated to dryness. The sample was left out until it was cold. The residue of each sample was added to 1 mL of distilled water and well dissolved. Then, 4 mL of anthrone reagent (0.2 g anthrone in 100 mL (H_2SO_4)) was added to the solution, then boiled in a water bath for 10 min. After cooling, soluble sugar contents were determined at 620 nm against blank.

2.5 Determination of vitamin C content

The modified Jagota and Dani method [21] was applied for vitamin C determination. Fresh samples were used, cut into small pieces, and weighed 7 g. Then, the samples were homogenized and suspended in 30 mL of oxalic acid (0.5% w/v). These homogenous mixtures were filtered through No. 40 Whatman filter paper, followed by adding 4 mL of 10% Trichloroacetic acid into 1 mL of the homogenous mixture, which was then shaken well and immediately placed on ice for 5 min. The mixture was centrifuged at 8,000 rpm for 5 min. Then, 0.2 mL of 0.2 M Folin-Ciocalteu's reagent was added to 3 mL of supernatant, and the mixture was left at room temperature for 60 min. The samples were measured at an absorbance of 760 nm with a spectrophotometer (Biochrom, Libra S12, England).

2.6 Determination of phenolic content

As in a previous study, total phenolic contents were determined using Folin–Ciocalteu's method [22], modified for the 96-well plate assay [23]. Fresh lettuce leaves were washed with tap water and repeated with distilled water. Then, the sample was cut into small pieces and ground into a fine paste. The sample was weighed at 5 g into a Duran bottle, and then 10 mL of 70% EtOH (v/v) was added and shaken for 1 h on a shaker plate. After that, sample extracts were filtered and rinsed into 1.5 mL microtubes and stored at -20 °C in the dark until further analysis. Folin–Ciocalteu's reagent (25 µL, 50% v/v) was added to 10 µL of extract (1 mg/mL, w/v) in a well of a 96-well plate. After 5 min of incubation at room temperature, 25 µL of 20% (w/v) sodium carbonate was added to the mixture, followed by distilled water to a final volume of 200 µL per well plate. After 30 min of incubation at room temperature, the absorbance was read at 760 nm against a blank using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). A standard curve was plotted using gallic acid [24].

2.7 Statistical analysis

This study's statistics were carried out using a CRD. Data analysis was done using the MS Excel program and one-way analysis of variance (ANOVA) (version 7.0). Treatment means underwent Least Significant Difference (LSD) analysis with a 95% confidence level.

3. Results and Discussion

3.1. Nitrate content

In a hydroponic vegetable farm, nitrates are assimilated through the root hair, xylem, and mesophyll cells, as they can pass through the cell wall via transporters. The result of hydroponic lettuce grown under the pre-harvest condition of replacing nutrient solution with tap water showed that nitrate content was decreased by 19 to 63% when compared with 0 h (51 mg/g dry weight, control treatment) (Fig. 1). The treatment of replacing nutrient solution with tap water for 72 h (19 mg/g dry weight, 63% reduction) showed the lowest nitrate content, followed by treatments of 48 h (20 mg/g dry weight, 61% reduction), and 24 h (41 mg/g dry weight, 19% reduction). However, the treatments between 72 h and 48 h did not show a statistically significant difference. Furthermore, the treatments at 0 h and 24 h did not show a significant difference from each other (Fig. 1). The results of this study are consistent with Guffanti et al. [25], who reported that reducing the concentration of the nutrient solution by adding water or completely substituting the nutrient solution with water in the last days before harvest can help reduce the amount of nitrate in leaves. The nitrate content was decreased by more than half of what it was at 48 h and 72 h, whereas that at 24 h treatment was not significantly reduced. The statistic suggested that pre-harvest treatment by replacing nutrient solution with tap water for 48 h was enough to decrease nitrate content. It is proposed that tap water clean up the residual nitrate in the xylem vessels of the roots, stems, and leaves of hydroponic green oak lettuce within 48 h. On the other hand, the electrons from the photosynthetic process play a key role in reducing nitrate assimilation to glutamate acid in plant cells, which could be another reason for less nitrate accumulation after pre-preharvest for 48 h [26]. However, Qiu et al. [8] have reported that during nitrate assimilation to glutamate acid in lettuce plants, some nitrate residue is in the xylem vessels of the root, stem, petiole, and leaf veins. This experiment was designed by replacing the nutrient solution with tap water to clean up the xylem vessels of the roots, stems, and leaves of hydroponic green oak lettuce. Furthermore, these results are similar to those of Guffanti et al. [25], Wanlai et al. [13], and Cometti et al. [27].

3.2. Vitamin C content

Plant vitamin C has an important antioxidant function and is a major source in the human diet. The hydroponic lettuce grown under the pre-harvest treatment of replacing nutrient solution with tap water for 72 h (5.3 μg/100 g, 30% reduction) showed the lowest content of vitamin C, followed by treatments of 48 h (6.3 μg/100 g, 16% reduction), and 24 h (7.2 μg/g dry weight, 4% reduction) (Fig. 2). The vitamin C content was decreased by 4 to 30% when compared with 0 h (7.6 μg/100 g, control treatment). The amount of vitamin C gradually reduced as the treatment duration increased, implying that vitamin C is most likely used during pre-harvest periods to convert H_2O_2 to H_2O [28,29]. H_2O_2 can cause the ROS reaction, activating the plant cells' stress defense mechanism. Vitamin C can reduce and control the appropriate H_2O_2 content in plant cells [28,29]. Vitamin C biosynthesis begins with the photosynthesis system that produces D-glucose-6-P, and vitamin C is stimulated in the mitochondria, then released and accumulated in the cytosol. This experiment showed that vitamin C content decreased after pre-harvesting when the nutrient solution was replaced with tap water. The reason is probably, the vitamin C content is accumulated in the cytosol before the pre-harvest experiment. Following the investigation, lettuce may use vitamin C to reduce H_2O_2 to H_2O [28-29], where H_2O_2 is normally induced by pre-harvest stress [30].

3.3. Phenolic content

The accumulation of phenolic content in plants indicates plant response to abiotic and biotic stresses. The phenolic content was not significantly different among the different time intervals under pre-harvest of replacing nutrient solution with tap water and control treatment. The treatment of replacing the nutrient solution with tap water for 24 h (125 μg/100 g, 13% reduction) showed the lowest phenolic content. It was followed by both treatments of 48 h and 72 h (both 134 μg/100 g, 6% reduction) (Fig. 3). The phenolic content was decreased by 6 to 13% when compared with 0 h (143 μg/100 g, control treatment). Still, there were no statistically significant differences among treatments. This result was consistent with lettuce and rocket plants grown in a vertical farm in a controlled environment hydroponic system [25]. As a result, this result indicates that pre-harvest nutrient solution replacement with tap water within 72 h was not stressful and had no adverse effect on crop quality. Phenolic biosynthesis in plants is triggered by H_2O_2 [30]. However, H_2O_2 was probably reduced by vitamin C as vitamin C in this experiment was decreased for use in the reduction of H_2O_2.

3.4. Soluble sugar content

Soluble sugars are highly sensitive to environmental stresses and act as nutrient and metabolite signaling molecules that affect plant cellular modification levels [31]. In the present study, we measured the soluble sugar of hydroponic lettuce grown under the pre-harvest condition of replacing the nutrient solution with tap water. The soluble sugar content ranged from 1.6 to 3.3 mg/100 g among treatments (Fig. 4). It significantly increased by 86 to 105% compared to 0 h (1.6 mg/100 g, control treatment). The treatment of replacing nutrient solution with tap water for 48 h (3.3 mg/100 g, 105% increase) showed the highest content, followed by treatments of 72 h (3.2 mg/100 g, 98% increase) and 24 h (3.0 mg/100 g, 86% increase). However, soluble sugar content did not significantly differ between treatments lasting 48 h and 72 h. This study showed that soluble sugar content is significantly increased after replacing nutrient solution with tap water in the uncontrolled environment of a hydroponic farm. It could be possible that energy sources for photosynthesis, such as ATP and $NAPH_2$, are not necessary for nutrient uptake, transportation, and stimulation of the nitrate molecule to glutamine acid. Instead, this energy was accumulated and may be changed to produce soluble sugar [26, 31]. This result was in contrast with the vertical farming technique of a hydroponic system, in which total sugar content did not significantly differ after replacing the nutrient solution with tap water [25].

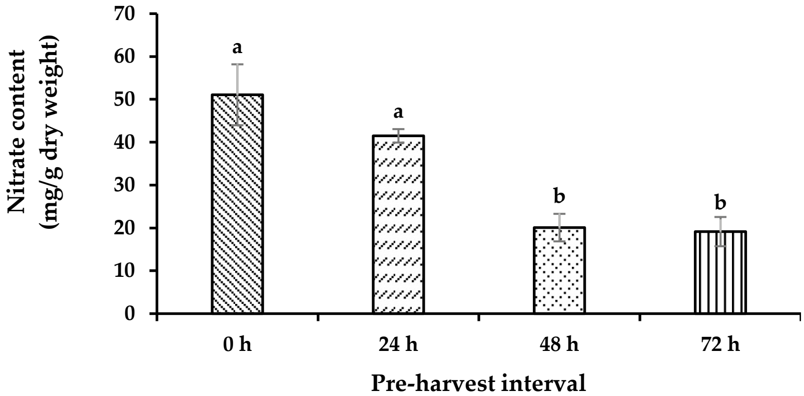

Figure 1. The effect of pre-harvest periods caused by replacing nutrient solution with tap water on nitrate content in hydroponic lettuce. The error bars display the standard error over three replications. The means indicated by the different letters are substantially different at $p < 0.05$, according to the least significant difference.

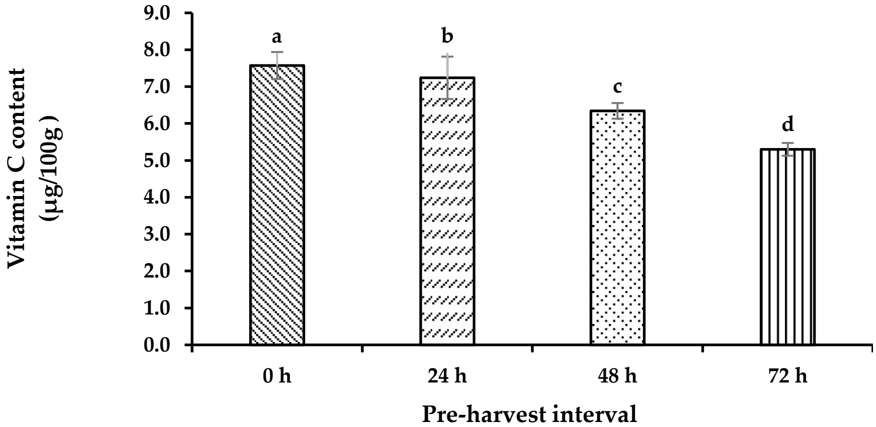

Figure 2. The effect of pre-harvest periods caused by replacing nutrient solution with tap water on vitamin C content in hydroponic lettuce. The error bars display the standard error over three replications. The means indicated by the different letters are substantially different at $p < 0.05$, according to the least significant difference.

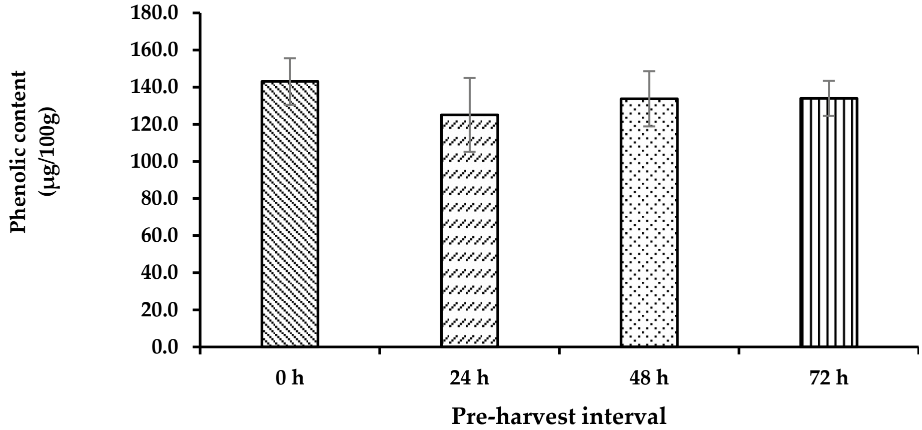

Figure 3. The effect of pre-harvest periods caused by replacing nutrient solution with tap water on phenolic content in hydroponic lettuce. The error bars display the standard error over three replications. The means indicated by the different letters are substantially different at $p < 0.05$, according to the least significant difference.

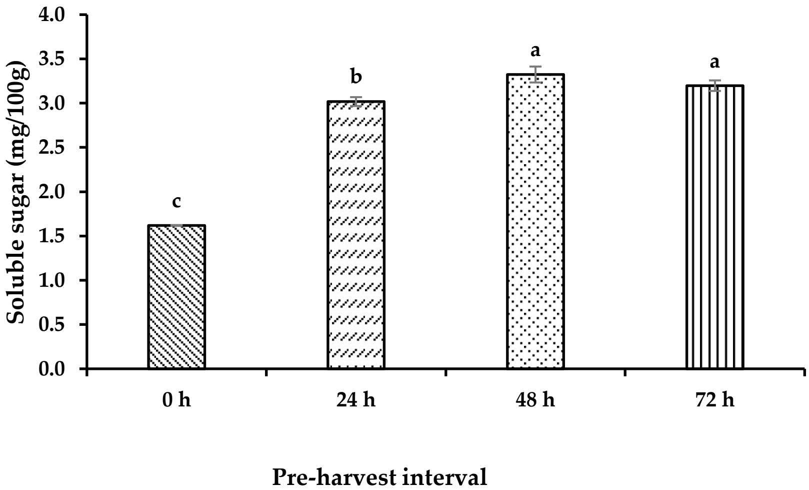

Figure 4. The effect of pre-harvest periods caused by replacing nutrient solution with tap water on soluble sugar content in hydroponic lettuce. The error bars display the standard error over three replications. The means indicated by the different letters are substantially different at $p < 0.05$, according to the least significant difference.

4. Conclusions

This study focused on the effect of pre-harvest periods by replacing nutrient solution with tap water on nitrate and quality in hydroponic lettuce. The results suggested that nitrate contents were reduced the lowest and soluble sugar contents were increased the highest after pre-harvest for 48 h. Vitamin C and phenolic contents were slightly decreased after pre-harvest periods by replacing the nutrient solution with tap water.

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Conflicts of Interest: The authors declare no conflict of interest.

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