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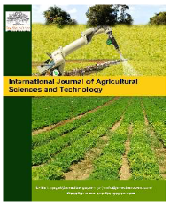

Research Paper

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# Growth, mineral content and antioxidant activity of romaine lettuce in relation to development stage in soilless system

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## Abstract

Lettuce is an important vegetable crop worldwide that widely grown hydroponically and usually consumed raw as salad. Developing an optimal harvest strategy could increase lettuce production and nutritional quality. The aim of the present research was to evaluate growth, mineral content, total phenols and flavonoids content and antioxidant activity of soilless (hydroponic) grown romaine lettuce (*Lactuca sativa* L. var. *longifolia*) leaves at three growth stages ("S1"-28 days after planting (DAP), "S2"-42 DAP, "S3"-56 DAP). Head fresh weight and number of leaves per head were higher at third growth stage; however, at this stage the size of lettuce head and leaf tissue hydration is highest which might affect the consumer choice for the produce. Regarding leaf chemical composition, the highest content of total phenols, flavonoids and antioxidant activity were observed during the first two growth stages. Mineral content of leaves generally decreased during plant development with significant highest contents being observed during the first growth stage for N and during the first two growth stages for P, K, Ca and Mg. In conclusion, the results of this study indicate that chemical composition of lettuce is highly dependent on plant development stage, and harvest stage might be considering for product biomass and bioactivity content to attain consumer's acceptance.

***Keywords:*** *Antioxidant capacity, Hydroponics, Phytophenoles, Romaine lettuce*



## 1. Introduction

In recent years, there had been an increased focus on vegetable production and marketing with regard to quality and produce safety (Hewett, 2006; Kader, 2008; Petropoulos *et al*., 2018). Romaine lettuce (*Lactuca sativa* L. *var. longifolia)* is an important leafy vegetable all over the world, which normally consumed raw as salad or as ready-to-eat produce. Its leaves are rich source in health-promoting bioactive compounds that have a positive effect on health, e.g., phenolic compounds, carotenes, ascorbic acid, and flavonoids (Llorach *et al*., 2008; Kim *et al*., 2016; Petropoulos *et al*., 2018). Lettuce leaves is also a good source of minerals, such as calcium (Ca), phosphorous (P), magnesium (Mg), and potassium (K) (Conversa *et al*., 2004; Kim *et al*., 2016).

Hydroponic (soilless) culture nowadays has became a popular cultivation system for producing vegetables especially lettuce due to many advantages like potential for higher yields per unit area with minimized use of water and it also

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provides a year-round source of fresh vegetables in human diets (Matthew *et al*., 2011; Al-Karaki *et al*., 2009). However, as consumer preferences for normally consumed raw or ready-to eat vegetables are driven by their visual appearance of product (e.g., mini-sized heads of lettuce) which might push farmers to harvest head lettuce at a smaller and immature stage (Gil *et al*., 2012). Therefore, we need to have a successful strategy of cultivation (e.g., optimum growth stage) that enhance biomass, chemical composition and nutritional value and attain consumer's acceptance.

Several studies have reported that the nutritional value of leafy vegetables is varied with developmental stages of growth (Petropoulos *et al*., 2018; Gil *et al*., 2012; Yosoff *et al*., 2015). Despite its nutritional importance, the nutrients content and phytochemicals of hydroponic grown lettuce have received less attention in relative to different plant development stages.

The main objectives of this study were to determine fresh and dry weights/plant, number of leaves/plant, total phenols, antioxidant activity and major minerals content in romaine lettuce grown hydroponically in greenhouse at three growth stages.

## 2. Materials and methods

### *2.1. Plant material and growing conditions*

Three-weeks-old seedlings of green cultivar of romaine (*Lactuca sativa* L. *var. longifolia*) lettuce that obtained from a local nursery in Irbid, Jordan, were used in this study. Lettuce seedlings were transplanted into soilless media (tuff zeolite: perlite, 1:1, V: V) filled in wood beds (1.4m L*1.0 m W*0.25m D). The wood beds were lined with plastic sheets for protection from moisture before fill with media, rose above soil level on metal frames and elevated at 1.5% for collection of drained water out of irrigation and transferred it down into irrigation tank for reuse in a closed re-circulating system. All plants were irrigated via drip irrigation system with a modified complete Hoagland nutrient solution (irrigation water and nutrients) from irrigation tank twice a day (15 min each time) with using timer to control the timing of irrigation. The nutrient solution contains (in mg/L): 210 N, 31 P, 230 K, 160 Ca, 48 Mg, 60 S, 0.5 B, 0.6Mn, 0.05 Zn, 0.02Cu, and 0.01Mo. The nutrient solution was renewed every two weeks. Plants were grown during the period December 2017- February 2018 under natural light in unheated greenhouse at Experimental farm of Faculty of Agriculture of Jordan university of Science & Technology, Irbid, Jordan. Cultivation was carried out at a planting density of 13 plants $m^{-2}$ (0.25m between plants and 0.30 m between rows). The ambient temperatures during the growing period were 23 ± 3 ºC/11 ± 2 ºC (day/ night) and relative humidity was 60-75%.

### *2. 2. Plant harvest and growth measurements*

Lettuce plants were harvested at three stages of plant development ["S1"-28 days after planting (DAP), "S2"-42 DAP and "S3"- 56 DAP]. After harvest, plants were immediately transferred to the laboratory for fresh weight determination. After taking fresh weights, three heads were taken randomly from each treatment and their leaves were separated for counting (e.g., >4 cm was counted) and then two groups of leaves were selected at random for use in chemical analysis. The first group of leaves was spreaded on trays for air-drying at room temperature for about 10 days, and the second group of leaves was placed in a forced-drying oven at 65°C for 4 days. Subsequently, the dry weights were determined and the material from both groups was stored at room temperature until further analysis. Moisture contents (leaf tissue hydration) were calculated for oven-dried leaf samples at three growth stages. Moisture content (%) was calculated using the following equation:

*Moisture content* (%) = *Fresh weight – Dry weight / Fresh weight* * 100

### *2.3. Determination of total phenols and flavonoids content and antioxidant activity*

For evaluation the contents of total phenols and total flavonoids and antioxidant activities, a representative sample from air-dried lettuce leaves were ground to powder pass through 0.5 mm sieve using a cyclone laboratory mill. Leaf samples were extracted in solvents (methanol/water) for chemical analysis according to the method described by Llorach *et al.* (2008). The total phenolic content of the leaf solvent extracts was determined according to the Folin - Ciocalteu method as described by Singleton *et al*. (1999). Gallic acid was used as calibration standard to produce the calibration curve and the concentration of total phenolics content was expressed as of Gallic acid equivalent (GAE) (mg /100 g dry weight basis). The total flavonoids content of the lettuce leaves extract was determined by the method described by Zhishen *et al*. (1999). Catechin was used as calibration standard and the concentration of total flavonoids was expressed as Catechin equivalent (CE) (mg CE/100g dry weight).

The antioxidant activity of lettuce leaves extract was evaluated according to their ability for scavenging free radicals by using the 2, 2- diphenyl-1-picrylhydrazyl (DPPH) method according to the procedures of Brand-Williams *et al.* (1995). Its absorbance was measured at 517 nm with a spectrophotometer (UV-VIS Spectroscan 50, Biotech Engineering Management Co., UK). The radical scavenging activity was calculated using the following equation:

$$Scavenging\ (\%) = (Ab - As/Ab) \times 100$$

Where *Ab* is absorbance of the blank (DPPH alone) and *As* is the absorbance of extract sample.

### 2.4. Determination of mineral content

Representative samples from oven-dried lettuce leaves were selected, ground to powder to pass through 0.5 mm sieve using a cyclone laboratory mill. The ground material was mixed thoroughly, and samples of 1.0 g were ashed for five hours at 550 ºC in a muffle furnace, and then the ash was dissolved in 2N HCl for determination of the concentration of P, K, Mg and Ca. Analyses were performed according to procedures of standard methods of analysis. The P was determined using spectrophotometer (Watanabe and Olsen, 1965); K and Ca by flame photometer (Ryan *et al*., 2001), Ca and Mg by Atomic Absorption Spectrometer (Varian AA 240 FS). The N content was determined using Kjeldahl's method.

## 3. Experimental design and statistical analysis

The experimental design was completely randomized design with three replications. Data was statistically analyzed using analysis of variance (ANOVA) and the mean values were compared using LSD test at 5% probability level.

## 4. Results and discussion

### 4.1. Plant growth

The average plant weights (both fresh and dry) and number of leaves per plant were significantly influenced by harvesting stage when these attributes increased with plant development and their highest values attained at the third growth stage (56 DAP) (Table 1). Harvesting at 42 and 56 DAP resulted in 142 and 250% higher lettuce fresh weight compared to early harvesting at 28 DAP (Table 1). Similar trends had been noticed by other researchers in many leafy vegetables, which they gain weight by continuously forming new leaves in the meristem apex (Palaniswamy *et al*., 2004; Petropoulos *et al*., 2005; Yosoff *et al*., 2015). Considering that leafy vegetables are usually intended for raw consumption, harvest stage is of high importance for marketability of product, since visual appearance might affect the consumer's preference of the product. However, harvest at early stage might give a high visual quality of leaves, but they do not reach the economic marketable size; while, harvest at late stage might affect the product quality and shelf life (Gent, 2012). Leaf tissue hydration (moisture content) varied with development stage of plants as the highest value of leaf hydration attained at the third growth stage (Table 1). Gent (2012) reported that high hydration in leafy vegetables might affect the quality attributes such as texture and visual appearance due to faster dehydration especially in larger heads of lettuce.

**Table 1: Head fresh and dry weights and number of leaves of romaine lettuce plants in relation to growth stage**

| Growth stage[1] | Head fresh weightg/plant | Head dry weight g/plant | Number of leaves/head | Leaf hydration % |
|---|---|---|---|---|
| S1 | 141.9c* | 11.4c | 13.4b | 92.0b |
| S2 | 344.1b | 21.7b | 19.6a | 93.8ab |
| S3 | 497.4a | 24.8a | 23.4a | 95.2a |

**Note:** * Means of the same column followed by different letters are significantly different according to LSD ($p < 0.05$); [1] Growth stages: S1 = 28 DAP; S2 = 42 DAP; S3 = 56 DAP.

### 4.2. Phytophenoles content and antioxidant activity

Phytophenoles (total phenols and flavonoids) contents and antioxidant activity were affected by growth stage (Table 2). Antioxidant activity of lettuce leaves was highest at the first two growth stages which are highly associated with increased content of total phenols and flavonoids (Table 2). Considering that harvests at medium growth stages may have a positive effect on product biomass and bioactivity content of leaves, these suggest that the second growth stage (42 DAP) as the most appropriate stage for harvest. Similar results have been reported in other leafy vegetables which indicated a high association between antioxidant activity and content of bioactive compounds such as phenolic compounds at different growth stages (Petropoulos *et al*, 2005; Brieudes *et al*., 2016).

**Table 2: Total phenols and flavonoids contents and antioxidant activity in leaves of romaine lettuce plants in relation to growth stage**

| Growth stage[1] | Total phenols mg /100 g DW | Flavonoids mg /100 g DW | Antioxidant activity (%) |
|---|---|---|---|
| S1 | 1322a* | 726a | 54.8ab |
| S2 | 1297a | 734a | 59.3a |
| S3 | 1074b | 541b | 46.5b |

**Note:** * Means of the same column followed by different letters are significantly different according to LSD ($p < 0.05$); [1] Growth stages: S1 = 28 DAP; S2 = 42 DAP; S3 = 56 DAP.

### *4.3. Mineral content*

Mineral content of lettuce leaves generally decreased during plant development with the highest contents being observed at the first two stages of growth (Table 3) especially for K, Ca and Mg, which are considered essential for enhancing product quality for human nutrition. Similar trend with varied growth stages of butter head lettuce were recorded for leaf mineral content (K, Ca and Mg) which attained the highest values at 22 DAP and then decreased at later growth stages before harvest (Conversa *et al*., 2004). The total N content was significantly higher at the first growth stage (28 DAP) than other two growth stages (42 and 56 DAP) of growth (Table 3). Conversa *et al*. (2004) reported that N content in hydroponically grown butter head lettuce leaves was increased at the early stages of growth up to 50 DAP and then decreased at later stage before harvest (60 DAP). Another important mineral for human health, leaf P content was found to be higher at the second growth stage (42 DAP) than other two growth stages (Table 3). Similar results had been noticed by other researchers in many leafy vegetables which indicated that P content at early stages of growth is lower than later stages before harvest (Conversa et al., 2004; Chukwu, 2013).

**Table 3: Mineral composition (mg/g) of romaine lettuce leaves in relation to growth stage**

| Growth stage[1] | N (%) | P (mg/g) | K (mg/g) | Ca (mg/g) | Mg (mg/g) |
|---|---|---|---|---|---|
| S1 | 3.46a* | 4.58ab | 65.9a | 14.8a | 4.87a |
| S2 | 3.12b | 5.03a | 60.4ab | 14.4a | 3.36ab |
| S3 | 2.82b | 4.22b | 58.9b | 13.0b | 2.46b |

**Note:** Means of the same column followed by different letters are significantly different according to LSD ($p < 0.05$); [1] Growth stages: S1= 28 DAP; S2= 42 DAP; S3= 56 DAP.

## 5. Conclusion

Plant growth and yield of romaine lettuce grown in soilless system is highly affected by harvesting growth stage, when fresh weight and number of leaves increased with plant development and their highest values attained at the third growth stage (56 DAP). However, the chemical composition of lettuce leaves was varied with developmental stages of growth. Considering that leafy vegetables are usually intended for raw consumption, therefore, harvest stage might be considered for product biomass and bioactivity content and attain consumer’s acceptance (visual appearance).

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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