Bragantia
ISSN: 0006-8705
editor@iac.sp.gov.br
Instituto Agronômico de Campinas
Brasil

Yosoff, Siti Fairuz; Muda Mohamed, Mahmud Tengku; Parvez, Anwar; Hajar Ahmad, Siti; Mohd Ghazali, Farinazleen; Hassan, Hamizah

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Bragantia, vol. 74, núm. 3, julio-septiembre, 2015, pp. 322-330
Instituto Agronômico de Campinas
Campinas, Brasil

http://dx.doi.org/10.1590/1678-4499.0453

Production system and harvesting stage influence on nitrate content and quality of butterhead lettuce

Siti Fairuz Yosoff ([1]); Mahmud Tengku Muda Mohamed ([2]); Anwar Parvez ([3*]); Siti Hajar Ahmad ([2]); Farinazleen Mohd Ghazali ([4]); Hamizah Hassan ([2])

([1]) University Pendidikan Sultan Idris, Agricultural Science Department, 35900 Tanjong Malim, Perak, Malaysia.

([2]) University Putra Malaysia, Institute of Tropical Agriculture, Department of Crop Science, 43400 Seri Kembangan, Selangor, Malaysia.

([3]) Bangladesh Agricultural University, Department of Agronomy, 2202 Mymensingh, Bangladesh..

([4]) University Putra Malaysia, Food Science Department, 43400 Seri Kembangan, Selangor, Malaysia.

(*) Corresponding author: parvezagron@yahoo.com

***Received:** Dec. 17, 2014; **Accepted:** Feb. 26, 2015*

Abstract

Leafy vegetables such as lettuce grown under different production systems may accumulate different concentrations of nitrate which may reach to the levels potentially toxic to humans. Moreover, nitrate accumulation varies in various plant parts and physiological age of the plant. Therefore, to determine the effect of production system and harvesting stage on nitrate accumulation and quality of butterhead lettuce, a study was conducted considering two lettuce production systems namely hydroponic and organic, and four different harvesting stages such as 35, 38, 41 and 44 days after transplanting (DAT). The experimental design was complete randomized design (CRD) with four replications. Hydroponic and organic systems performed similar in terms of yield, quality and nitrate content of butterhead lettuce. Delaying harvesting can not only increase yield but also can minimize nitrate accumulation and health hazard risk as well. Delay in harvesting stage may result in quality deterioration of lettuce and increased production cost. Thus, a compromise is necessary to consider 41 DAT as the optimum stage to harvest butterhead lettuce with significantly higher reduction of nitrate content in both outer adult leaf blades and young leaves of hydroponic lettuce. Fresh weight, firmness and color of butterhead lettuce at this stage were still acceptable.

Key words: hydroponic, organic, harvesting time, nitrate content, butterhead lettuce.

1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a leafy vegetable from Asteraceae family, and the only member of the *Lactuca* genus grown commercially (Koike et al., 2007). It is a good source of different vitamins and minerals especially vitamin A and potassium. As reported by FAO (2010), world production of lettuce in 2010 was more than 23 million tons, and those were primarily from China (53%) and the United States (17%).There are several types of lettuce, but most common are the Leaf, Head and Cos or Romaine lettuce (Katz & Weaver, 2003). Butterhead lettuce, a head type lettuce, forms open heads with softer leaves and has much smoother and delicate texture (Bradley et al., 2010). Butterhead lettuce is one of the most popular varieties in Western Europe, where it accounts for about 80% of lettuce consumption.

Vegetable production and marketing have received increasing attention with regard to quality and safety of produce (Hewett, 2006; Kader, 2008). In recent years, there had been an increased focus on food safety all over the world. Public food safety standards have been enforced through legislation, and business firms at different degrees of the furnish chain have developed various private standards (Hammoudi et al., 2009). Legislations adopted to improve food safety include standards regarding the characteristics of the final product, production practices in supply chain, traceability within the supply chain and legal liability of the supply chain. Lettuce is normally consumed raw as salad. Thus, it is prone to food safety risk if nitrate contamination exceeds the recommended limit. Nitrate is one of the major sources of N available to higher plants including vegetables. However, over fertilization which is usually the case may cause high nitrate accumulation in plants, especially leafy vegetables. High concentration of nitrate in the edible part of the plant may be implicated in the occurrence of methaemoglobinemia or blue baby syndrome and possibly in gastric cancer as well as other diseases (Ikemoto et al., 2002; Ishiwata et al., 2002).

Leafy vegetables grown under different production systems may accumulate different concentrations of nitrate which could reach potentially harmful concentrations. Nitrogenous fertilizers, mainly of nitrate base are used widely in vegetable production if the rate of its uptake exceeds the rate of its reduction to ammonium then nitrate will start to accumulate. In general, higher rates of nitrate application increase the plant nitrate content without increasing the yield. Therefore, growers who apply excessive fertilizers to ensure that nitrogen is not limiting for plant growth are unlikely to achieve any gain in terms of yield but will only increase the nitrate content of crops to the levels potentially toxic to humans.

Nitrate in the plant is either being reduced or stored in the vacuoles. It is also being transported in the xylem transpiration stream to the leaf for reduction. However, mostly it is stored in the vacuole until released for a reduction in the cytosol. In addition, nitrate reductase (NR) exists in the cytosol and nitrate in the cytosol is called nitrate metabolic pool. On the other hand, nitrate in the vacuole is called nitrate storage pool (Miller & Smith, 1996). NR was assumed the rate-limiting factor for nitrate assimilation. NR is an inducible enzyme and thus, there is a close relationship between nitrate reductase activity (NRA) and nitrate concentration in plants. Accumulation of nitrates in lettuce also has been shown to be affected by the soil texture and the source of fertilizer-N (Gunes et al., 1995). The nitrate accumulation varies in various part of plant and physiological age of the plant. In most types of lettuce, including butterhead type, the highest concentration of nitrates is normally observed in the external leaves (Abu Rayyan et al., 2004). The NRA also varies with plant structures especially the leaf. However, whether production system and leaf structures influence the NRA is still uncertain. Therefore, there is a need to understand the effects of production systems, harvesting stages and leaf structures on nitrate content and NRA in order to reduce the nitrate accumulation. A study was therefore conducted to determine the effect of production system and harvesting stage on the nitrate accumulation, nitrate reductase activity and quality of butterhead lettuce.

2. MATERIAL AND METHOD

Experimental site: According to Koppen classification system, the experimental site (3°00' 21.34" N, 101°42' 15.06" E, 37 m elevation) belongs to Tropical rainforest climate *(Af)* which is characterized by constant high temperature (18 °C or higher) throughout the year, average precipitation of at least 60 mm in every month and no natural seasons. During the experimental period, monthly average maximum and minimum temperature and relative humidity ranged from 33.7 to 34.5 °C, 22.9 to 23.4 °C and 94.2 to 96.3%, respectively, while rainfall, evaporation and sunshine hours ranged from 4.5 to 12.3 mm/day, 3.9 to 4.4 mm/day and 6.81 to 7.42 hrs/day, respectively.

Plant material: Butterhead lettuce (*Lactuca sativa* L.), a popularlettuce variety, was used as the plant material in this study. Butterhead lettuce is a head type lettuce with open heads ,softer leaves with much smoother and delicate texture.

Experimental design and treatments: The experiment was conducted using a complete randomized design (CRD) with a factorial arrangement of treatments and four replications. Treatments included two lettuce production systems namely hydroponic and organic, and four different lettuce harvesting stages such as 35, 38, 41 and 44 DAT. For nitrate content and nitrate reductase activity (NRA) determination, three different leaf parts namely midribs, outer adult leaf blades and young leaves were also considered as treatments.

Crop husbandry: Hydroponic butterhead lettuce was grown under a rain shelter and organic lettuce was grown in an organic-field plot. For hydroponic system, butterhead lettuce seeds were sown on a medium-wetted sponge (2.5 cm × 2.5 cm) at the TPU nursery. After two weeks, seedlings were transplanted into 20 L planting troughs containing 50 ml stock A and 50 ml stock B complete Hoagland nutrient solutions (Fertitrade (M) Sdn, Bhd., Malaysia) as liquid fertilizer. The water salinity, expressed in electrical conductivity (EC), was maintained at 1.3-1.5 EC throughout the plant growth. For the organic system, the seeds were sown into seedling trays (100 cells/tray) containing peat moss as substrate, in the TPU nursery. After two weeks, the seedlings were transplanted into raised planting beds, organic plots, at the rate of 1 seedling/hole, with each bed measuring 1 m wide x 1 m long and 20 cm high. The distance between beds was 0.5 m in a field plot of 11 m wide x 6.5 m long. The distance between planting rows on each bed was 20 cm and between plants within a row was 20 cm, giving a total of 25 plants per replication. General agronomic practices carried out in the field were weeding, watering, pest management and soil fertilisation. The plants were watered by sprinkler irrigation twice a day. Ten ton/ha chicken manure was incorporated into each bed, one week before transplanting. Organic compound NPK fertilizer (8: 8: 8) was also applied at the rate of 100 kg N/ha at 10 days after transplanting. The fertilizer was applied along the plants rows. At 35, 38, 41 and 44 DAT, both of the hydroponic and organic lettuce heads were harvested and immediately transported to the laboratory for analysis.

Plant height and fresh weight determination: Three lettuce plants were randomly selected from each replication for plant height and fresh wight determination. Plant height was measured from the surface of the growing media to the top of the lettuce head using a measuring tape. Then, the plant was removed from the growing media by cutting the basal stem with a sharp knife. Foreign matter and remains of the growing media sticking to the lettuce were washed off with water. The lettuce was blotted gently with a soft paper towel to remove any free surface moisture and then, immediately weighed using a weighing balance (Model B303-5, Mettler Toledo, Japan).

Firmness determination: Four outer leaves from each of head lettuce were randomly selected and the firmness of midrib was determined at two equidistant points from each leaf petiole. Measurement of firmness was made using an Instron Universal Testing machine (Model 5543, Instron Corp., USA), with a 6 mm diameter cylindrical probe at a speed of 20 mm min^{-1}. The Instron was used simultaneously with an Instron Merlin Software version M12-13664-EN for the processing analysis. Eight readings were made randomly on each lettuce head and the mean was calculated.

Color measurement: Color of leaves was measured randomly on three outer layer leaves per head by a chromameter (Model CR-300, Minolta Corp., Japan) using Illuminate C (CIE 1976) with an 8 mm measuring head and calibrated with a standard white tile. Before measurements were made, the instrument was calibrated against a standard white plate with a standard value of Y=93.2, x=0.3136 and y = 0.3195. For accuracy of the reading, the measuring head was cleaned everytime before use. Color was measured as lightness (L*), chroma (C*) and hue angle (h°) of CIE-lab scale. The L* value indicates the lightness, ranged from 0= black to 100= white. The a* and b* values were used to determine the hue angle (h°) and chromaticity (C*). The C* measured the vividness of colors and obtained by $C^*=(a^{*2}+b^{*2})^{0.5}$. The h° is the actual color or perceived color used to classify the kind of color which vary continuously from 0° to 360°. The h° is arc tan (b*/a*) that 0°/360° corresponded to red, while 90°, 180° and 270° indicate yellow, green and blue, respectively.

Nitrate content determination: Lettuce extract were obtained from three different leaf parts on each lettuce; four young leaves from the inner leaf layers, four adult leaf blades from the outer leaf layers and four midribs from each outer adult leaf blades. Each part was chopped separately and only 20 g from chopping lettuce was grounded finely using a pestle and mortar. The paste was filtered through cotton wool to get the cell sap. Nitrate content of the cell sap was determined using a nitrate meter (Cardy Twin Nitrate Meter, Spectrum Technologies Inc., USA) as described by Hochmuth (1994). The glass electrode of the meter was calibrated with buffers at 2000 and 150 mg kg^{-1} NO_3 before use. After calibration, the glass electrode was washed with distilled water and wiped with a soft tissue paper. Three drops filtrate of cell sap was dropped on the electrode of nitrate meter and a stabilized nitrate reading was recorded. The concentration of nitrate was expressed in mg kg^{-1} of fresh weight (FW).

In vivo nitrate reductase activity (NRA) determination: Chopped lettuce sample (same as nitrate content determination) of 0.3 g each was placed in a test tube (16×150 mm) and added with 10 ml incubation medium containing 0.1 M potassium phosphate buffer (pH 7.5) of 0.1 M KNO_3 and 5% (v/v) isopropanol. The test tubes were sealed with rubber stoppers and incubated in a water bath for one hour in the dark room at 30 °C. After incubation, the samples were placed in a boiling water bath to stop the NRA. The samples were cooled at room temperature. Five millilitres sulphanilamide and 5 ml NED were added and mixed by a vortex mixer (Model SA7, Stuart, United Kingdom). After 10 min, the samples were measured by using a spectrophotometer (Model S1200, Spectrawave, England) with absorbance readings at 540 nm. The nitrite released to the medium was expressed as μmol NO_2^- h^{-1} g^{-1} FW.

Statistical analysis: The data were analyzed using ANOVA, and mean seperation was done by least significant difference (LSD) test at $p \leq 0.05$ (SAS Institute, 2003). Regression analysis was carried out when the interaction between factors was significant.

3. RESULTS AND DISCUSSION

Plant Height: Plant height of butterhead lettuce was significantly affected by harvesting stage, but not by production system. The interaction effect was also insignificant (Table 1). In both the production systems, lettuce height was around 13 cm. Plant height increased gradually with the delay in harvesting. Lettuce plant height was recorded 35 cm when harvesting was done at 44 DAT, which was statistically similar to that (32 cm) when harvested at 41 DAT. Shortest lettuce plant of only 27 cm was obtained when harvesting stage was 35 DAT. Lettuce plant harvested at 44 DAT was 28% taller than that harvested at 35 DAT.

Fresh Weight: Like plant height, lettuce fresh weight was also significantly affected by harvesting stage, but not by production system. There were no significant interaction between production system and harvesting stage regarding fresh weight of lettuce (Table 1). Lettuce fresh weight recorded under hydroponic and organic systems were 129 and 143 g, respectively. Lettuce fresh weight increased gradually with as harvesting delayed. Harvesting at 38, 41 and 44 DAT resulted in 32, 45 and 93% higher lettuce fresh weight compared to early harvesting at 35 DAT.

Many researchers reported varying performances of vegetables grown under different production systems. For example, Uddin et al. (2009) reported that organic plants fertilized with chicken manure had higher growth parameters and marketable yield compared with conventional plants. Xu et al. (2005) observed that vegetables grown with organic fertilizers performed better than those grown with chemical fertilizers. Magkos et al. (2003) also confirmed that organic planting system produced higher biomass and better quality of different vegetables including lettuce than conventional planting system. In the present study, production system did not affect plant growth in terms of plant height and fresh weight of butterhead lettuce. This probably could be due to the fact that we compared between organic and hydroponic systems, while in other studies the comparison was between organic and conventional systems. However, as expected, the plant height and fresh weight increased with a longer growing period. This could be due to natural plant growth and development in course of time. It is in agreement with the findings of Michael et al. (2010), who found the plant height and weight of red lettuce 'Veneza Roxa' were increased by the prolonging of the growing period.

Firmness: Firmness of butterhead lettuce was not significantly affected by production system, harvesting stage and their interaction (Table 1). Firmness was recorded 40 and 42 N under hydroponic and organic systems, respectively, and ranged between 36 and 43 N among different harvesting stages. Murphy et al. (2011) also found no significant changes with respect to texture character of lettuce between production systems. It was probably due to the sufficient availability of calcium in both production systems that could help maintain the lettuce firmness. For many vegetables including lettuce, firmness is one of the much desirable texture qualities that maintain their firm and crunchy texture and consumers associate these textures with the freshness and wholesomeness (Fillion & Kilcast, 2002).

Leaf Color: Leaf color of butterhead lettuce, in terms of chroma (C*), lightness (L*) and hue (h°) values, was significantly affected by production system, harvesting stage and their interaction (Table 2). Chroma (C*), lightness (L*) and hue (h°) values of lettuce leaves were found higher under organic system than under hydroponic system. All the leaf color parameters were recorded the highest when lettuce was harvested at 35 DAT. There were no significant differences in leaf color parameters among harvesting stages 38, 41 and 44 DAT (Table 2).

C* values of organic lettuce had no significant relationship with the harvesting stage (Figure 1). The C* was consistent throughout the harvesting stages. It was contrary with the hydroponic lettuce where C* was significantly affected by the harvesting stages. The C* values of hydroponic lettuce showed a positive and quadratic relationship with harvesting stages (R^2=0.91). This indicated that 91% variability of chroma values of hydroponic lettuce was affected by the harvesting stage. There was a quadratic decrease in C* from 35 to 41 DAT, then the C* values were slightly increased until 44 DAT. The lightness and hue angle values of hydroponic lettuce showed similar trends (Figures 2 and 3). Both L* and h° values of hydroponic lettuce showed significant, positive and quadratic relationships with the harvesting stage (R^2=0.76 and 0.70, respectively). This denoted that 76% of variability of L* values and 70% of variability of h° values were affected by stage of harvest. Greater L* values indicated a lighter color compared to smaller values for darker color (0 = black to 100 = white) while the hue angle values indicated the actual color of butterhead lettuce. Changes in the respective color values showed that the color of the hydroponic-grown lettuce also changed from light green to greenish yellow. These indicated the loss of vividness or saturation of color as the lettuce started to sense from 38 to 44 DAT. Yellowing, or loss of green color, normally

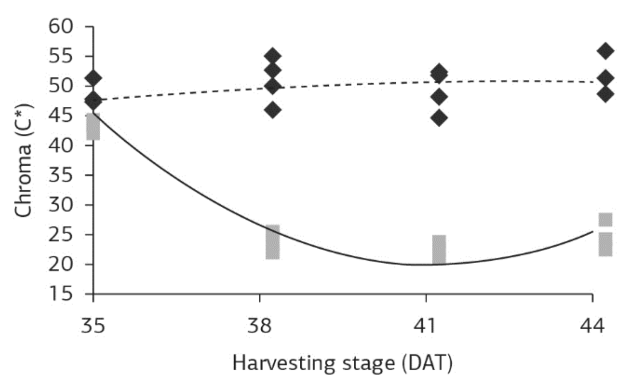

Figure 1. The relationship between chroma value and harvesting stage of hydroponic (■)= $45.28-8.22x+0.70x^2$ (R^2=0.91) and organic (♦) Butterhead lettuce. Solid line indicates a significant relationship at p≤0.05. Each dot represents four samples. DAT = Days after transplant.

Table 1. Main and interaction effects of production systems and harvesting stages on plant height, fresh weight and firmness of Butterhead lettuce

Factor	Plant height (cm)	Fresh weight (g)	Firmness (N)
Production system (P)			
Hydroponic	31.13 a[z]	129.66 a[z]	40.07 a[z]
Organic	31.63 a	143.38 a	42.79 a
Harvesting stage (D)			
35	27.63 c	95.75 c	36.02 a
38	30.13 bc	126.88 b	42.81 a
41	32.63 ab	138.81 b	43.03 a
44	35.13 a	184.63 a	43.86 a
Interaction			
P×D	ns	ns	ns
CV	15.03	18.41	19.52
SE	0.83	6.86	2.17

[z] Means with the same letters within a column and each factor are not significantly different using LSD test at p≤0.05. [ns] Not significant.

Table 2. Main and interaction effects of production systems and harvesting stages on the chroma, lightness and hue of Butterhead lettuce

Factor	Chroma (C*)	Lightness (L*)	Hue (h°)
Production system (P)			
Hydroponic	32.76 b[z]	42.31 b[z]	114.57 b[z]
Organic	49.74 a	51.89 a	124.70 a
Harvesting stage (D)			
35	46.79 a	51.60 a	125.66 a
38	37.92 b	45.97 b	115.44 b
41	37.41 b	45.70 b	117.67 b
44	36.11 b	45.13 b	119.76 b
Interaction			
P×D	**	*	**
CV	8.98	13.92	16.45
SE	1.31	0.68	1.09

[z] Means with the same letters within a column and each factor are not significantly different at p≤0.05 using LSD test. **, *, Significant at p≤0.01 and p≤0.05, respectively.

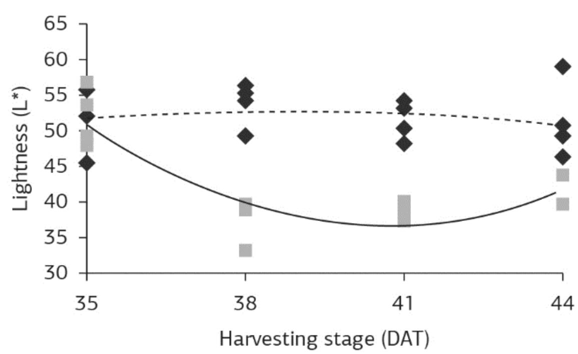

Figure 2. The relationship between lightness value and harvesting stage of hydroponic (■)= 51.08-5.04x+0.44x² (R^2=0.76) and organic (3) Butterhead lettuce. Solid line indicates a significant relationship at p≤0.05. Each dot represents four samples. DAT = Days after transplant.

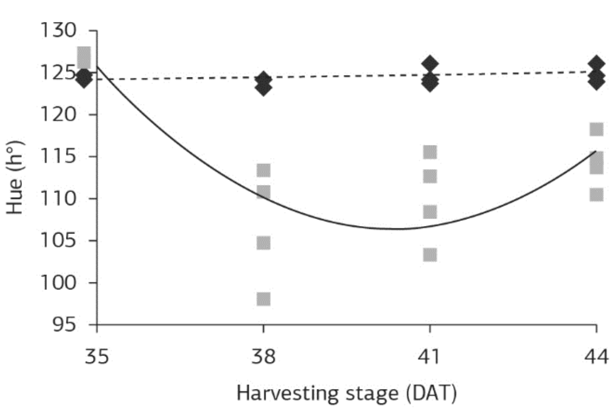

Figure 3. The relationship between hue angle and harvesting stage of hydroponic (■)= 125.7-7.20x+0.68x² (R^2= 0.70) and organic (♦) Butterhead lettuce. Solid line indicate a significant relationship at p≤0.05. Each dot represents four samples. DAT = Days after transplant.

considers the major consequence of chlorophyll degradation. Yamauchi & Watada (1991) reported that the loss of green color in parsley leaves was reflected by a reduction of chroma which was directly related to chlorophyll breakdown with pheophytin accumulation involved. The organic lettuce was seen as more resistant to color changes compared with hydroponic lettuce. This might be due to low pH in organic soil that could inactivate chlorophyll degrading enzyme magnesium dechelatase (Arkus et al., 2005) in the lettuce.

Nitrate Content: The effect of production system, leaf part and harvesting stage, and their interactions on the nitrate content of butterhead lettuce were significant (p≤0.01) (Table 3). In this study, nitrate content of butterhead lettuce was found much below than the maximum nitrate limit (MNL) for the European Commission . Nitrate content of lettuce was recorded 57% higher when grown under hydroponic system compared to organic system. Nitrate content in different leaf parts of lettuce followed such order; midribs>outer adult leaf blades>young leaves. Nitrate content of outer adult leaf blades and young leaves was respectively 50 and 58% lower than that of midribs. Nitrate content reduced gradually as harvesting of lettuce delayed. Nitrate content of lettuce harvested at 38, 41 and 44 DAT were 21, 35 and 37% lower than that harvested at early stage of 35 DAT (Table 3).

Nitrate accumulations in leaf structures of hydroponic lettuce were significantly different and followed such order; midribs>outer adult leaf blades>young leaves. In hydroponic lettuce, the nitrate contents were 52% and 75% lower in outer adult leaf blades and young leaves, respectively, if compared to midrib. Nitrate content in leaf structures of organic lettuce was also significantly different. Outer adult leaf blades were 22% and young leaves were 46% lower in nitrate content compared with midribs (Figure 4). As shown in Figure 5, nitrate contents of butterhead lettuce grown under hydroponic system showed a significant and a quadratic relationship with harvesting stages (R^2=0.85). This indicated that 85% of the variability in nitrate contents were affected by harvesting stage. Other factors that might affect the nitrate contents were light intensity and temperature. By comparing with 38 DAT, nitrate contents reduction in hydroponic lettuce was 24% when harvested at 41 DAT. However, there was no significant relationship between nitrate content in organic lettuce with harvesting stage. The results also showed that nitrate contents were below MNL for both the production systems. The relationships between nitrate content of lettuce midribs and harvesting stage were significant, positive and quadratic with R^2=0.64. In contrast, nitrate content in outer adult leaf blades and young leaves of lettuce showed significant, positive and quadratic relationships with harvesting stage (R^2=0.84 and R^2=0.93).

Table 3. Main and interaction effects of production systems, harvesting stages and leaf part on the nitrate content of Butterhead lettuce

Factor	Nitrate (mg kg^{-1} FW)
Production system (P)	
Hydroponic	2005.42 a^z
Organic	1278.75 b
Leaf part (L)	
Midribs	2569.06 a
Outer adult leaf blades	1279.69 b
Young leaves	1077.50 c
Harvesting stage (D)	
35	2145.83 a
38	1691.67 b
41	1385.00 c
44	1345.83 c
Interaction	
P×L	**
P×D	**
L×D	**
P×L×D	**
CV	63.92
SE	107.14

^{z} Means with the same letters within a column and each factor are not significantly different at p≤0.05 using LSD test. ** significant at p≤0.01.

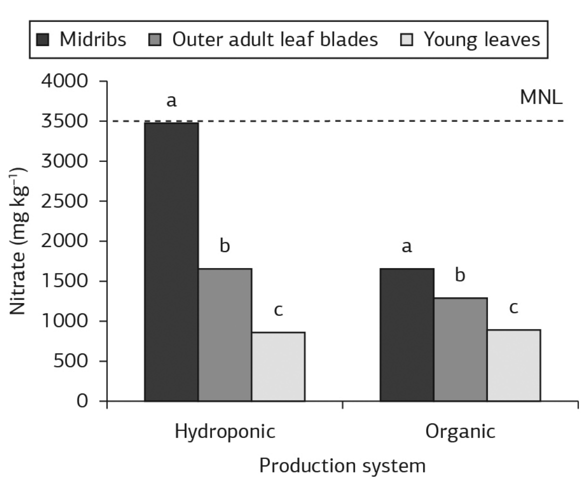

Figure 4. Nitrate content of Butterhead lettuce in leaf midribs, outer adult leaf blades and young leaves from hydroponic and organic production systems. Mean values between leaf parts within a production system followed by the same letter are not significantly different. MNL = Maximum nitrate limit.

If compared with 38 DAT, the highest reduction of nitrate in midrib was 29% at harvesting stage 44 DAT, in outer adult leaf blades was 20% at 40 DAT and in young leaves was 21% at 40 DAT (Figure 6).

In this study, the nitrate content in organic lettuce was far below the MNL. The nitrate uptake by the plants could be affected by the type of growing media used. Organic lettuce was grown in the organic soil that contained soil colloids. Soil colloids comprised organic soil particles that retain nutrients for release into the soil solution where they are available for uptake by the roots. Thus, the soil colloids serve to maintain a reservoir of soluble nutrients in the soil without luxurious nitrate consumption (Hopkins & Hüner, 2008). In this study, the nitrate content in hydroponic lettuce was

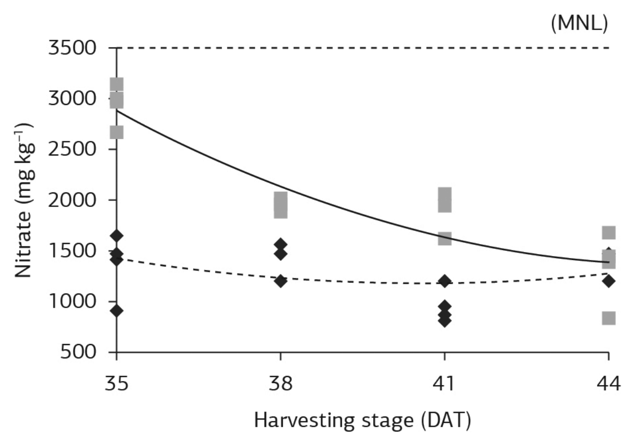

Figure 5. The relationship between nitrate content and harvesting stage of hydroponic (■)= 2880.50-297.19x+14.68x^2 (R^2=0.85) and organic (♦) grown Butterhead lettuce. Solid lines indicate significant relationships at p≤0.05. Each dot represents four samples. MNL = Maximum nitrate limit. DAT = Days after transplant.

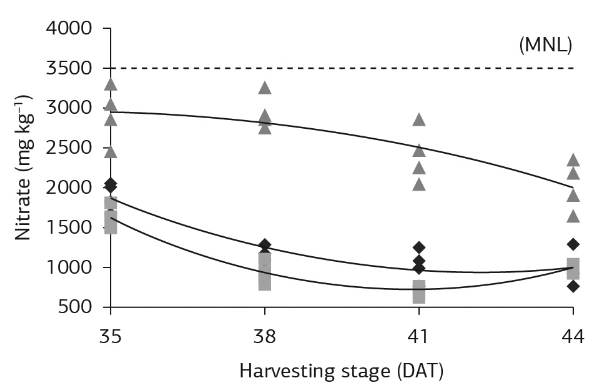

Figure 6. The relationship between nitrate content and harvesting stage in different leaf parts of Butterhead lettuce. Midribs (▲) = 2949.90-7.10x-11.08x^2 (R^2=0.64); outer adult leaf blades (♦) = 1878.90-265.15x+18.85x^2 (R^2=0.84); and young leaves (■) = 1626.6-309.67x +26.81x^2 (R^2=0.93). Solid lines indicate significant relationships at p≤0.05. Each dot represents four samples. MNL = Maximum nitrate limit. DAT = Days after transplant.

higher than organic lettuce. The major problems of hydroponic production system were selective depletion of ions and associated changes in the pH of the solution that occur as the roots continue to absorb nutrients. Lettuce maintained in pure solution culture would continue to grow vigorously if the nutrient solution is replenished on a regular basis. In order to avoid such problems, non-nutritive medium such as acid-washed quartz sand, perlite or vermiculite could be used to grow the plant. Then, the plants could be watered by daily application of a fresh nutrient solution either by slop or drip culture. Vogtmann et al. (1984) reported that spinach, Swiss chard, head lettuce and corn salad fertilized with composted farmyard manure had significantly lower nitrate content compared to mineral fertilizer. They stated that this was due to slow release of nutrient fertilizer. Several other studies also confirmed that the nitrate content in organically grown vegetables was lower than conventionally grown vegetables (Leclerc et al., 1991). It has been confirmed that the nitrate content in organically grown vegetables was lower than conventionally grown vegetables (Leclerc et al., 1991). Unregulated application of nitrogen fertilizers results in luxury consumption of nitrogen uptake by the plant, which may lead to accumulation of nitrate in leaves, thus reducing the quality of the produce. Despite the health benefits of organic vegetables, high nitrate level may pose a safety problem. The nitrate accumulation in hydroponic lettuce may also be due to high water content in lettuce. According to dynamic lettuce model NICOLET developed by Seginer et al. (2004), prediction of nitrogen uptake for the substantial nitrate pool of lettuce depends on the water content.

Lettuce-midrib tended to accumulate higher nitrate content than outer adult leaf blades and young leaves because of the nitrate assimilation pathway in plant. Nitrate is assimilated in the roots and also in the leaves (Hopkins & Hüner, 2008). When the capacity for nitrate assimilation in the roots reaches its maximum, nitrate is released from the roots into the xylem vessels and carried by the transpiration stream to the midribs, outer adult leaf and finally young leaves. In lettuce, midribs were the first place of leaf structure that got the nitrate after the roots. Hence, the tendency of nitrate accumulation in midrib was high. As confirmed by many researchers, nitrate content in different vegetable organs of plants followed the order like, petiole>leaf>stem>root> inflorescence>tuber >bulb>fruit>seed (Meah et al., 1994; Santamaria et al., 1999). Nitrate is mainly found in cell vacuoles and transported in the xylem. The xylem carries water and nutrients from the roots to the leaves while the phloem carries the products of photosynthesis from the leaves to the growing points of the plant (EFSA, 2008). One of the consequence of the transport system is that young leaves have lower nitrate concentration than outer adult leaf blades. In this respect, Greenwood & Hunt (1986) reported that nitrate concentrations in the outer leaves of cabbage were higher than inner leaf layers. Santamaria et al. (1999) also found that nitrate content differs in the various parts of a plant.

Delayed crop harvest may increase production cost and occupation of land for a longer period without any benefit. On the other hands, premature harvesting of many vegetables resulted in lower yield and quality as reviewed by Kader (2008). Thus, determining the suitable harvesting stage for each planting system of lettuce is particularly essential. The result shows that nitrate content was higher in the early plant growth stages and decreased with maturity. This was in agreement with Temme et al. (2010). Longer growth periods favor the reduction of nitrates except for young lettuce leaves which grow and expand to fully matured leaves. During initial growth, much of the nitrate taken up by the plant was used for roots and shoots development. At this stage, the roots were able to take up more nitrate than was required, and it accumulated in the stems and leaves of the plant. As the plants developed, the leaves were able to convert more nitrate into plant protein. Therefore, less nitrate is found in the plant as it matures, especially in outer adult leaf blades. The results showed that delayed harvest could lower nitrate accumulations but whether at the expense of texture or not needs further investigation.

Nitrate reductase activity (NRA): The NRA of butterhead lettuce was not significantly different between hydroponic and organic lettuce, but was different among various leaf parts. The interaction between production system and leaf part was also insignificant for NRA of lettuce (Table 4). In both the production systems, NRA was recorded 0.108 μmol $NO_2^{-1}h^{-1}g^{-1}$ FW leaves. Among the different plant parts, NRA was observed the highest in young leaves (0.111 μmol $NO_2^{-1}h^{-1}g^{-1}$ FW leaves), while midribs and outer adult leaf blades showed statistically similar NRA (Table 4). This seems logical because active photosynthetic activity and assimilation take place in young leaves, while midribs carry nitrate in the xylem sap from the roots. Thus, as pointed out by Carasso et al. (1998), nitrate distribution varied with tissues. It was also reported by Black et al. (2002) that the NRA was greater in leaves especially in young leaves.

Nitrate cannot be assimilated directly, but at first must be reduced to NH_4^+ in order to be assimilated into organic compounds. This is a two-step process, the first step is the the reduction of NO_3^- (nitrate) to NO_2^- (nitrite) by enzyme NR which is generally assumed to be a cytosolic enzyme. Later, NO_2^- moves into plastids (in roots) or chloroplast (in leaves) where it is quickly reduced to NH_4^+ by the enzyme nitrite reductase (NiR). Nitrite is toxic and rarely found at high concentrations in plants. Nitrate reductase has great influence on nitrate accumulation in plants. However, the relationships between NRA and nitrate concentration is still uncertain. Some studies indicated negative relationship between NRA and nitrate concentration (Hu et al., 1992). While other studies showed that, with NR being a substrate-induced enzyme,

Table 4. Main and interaction effects of production systems (hydroponic and organic) and leaf parts (midribs, outer adult leaf blades and young leaves) on the nitrate reductase activity of Butterhead lettuce

Factor	NRA (μmol $NO_2^{-1}h^{-1}g^{-1}$ FW leaves)
Production system (P)	
Hydroponic	0.108 a[z]
Organic	0.108 a
Leaf part (L)	
Midribs	0.107 b
Outer adult leaf blades	0.108 b
Young leaves	0.111 a
Interaction	
P×L	ns
CV	39.37
SE	0.04

[z] Means with the same letters within a column and each factor are not significantly different at p≤0.05 using LSD test. [ns] Not significant.

the higher substrate-nitrate concentration in plant led to higher NRA. So, there was a positive correlation between them (Ivashikina & Sokolov, 1997). Some investigations showed that a very small amount of nitrate is sufficient for induction (Matt et al., 2002) where NRA is not being induced when nitrate concentration was higher than a certain threshold level (Chen et al., 2004).

4. CONCLUSION

Hydroponic and organic systems performed similar in terms of yield, quality and nitrate content of butterhead lettuce. Therefore, either of the production system can be adopted depending on their availability and comparative advantages. Delaying harvesting can not only increase yield but also can minimize nitrate accumulation and simultaneously reduce the risk of health hazard to lettuce consumers. However, prolonged harvesting stage may result in quality deterioration of lettuce and increased production cost. Thus, a compromise is necessary to consider 41 DAT as the optimum stage to harvest butterhead lettuce with significantly higher reduction of nitrate content in both outer adult leaf blades and young leaves of hydroponic lettuce. Fresh weight, firmness and color of butterhead lettuce at this stage are still acceptable.

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