International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-7, Special Issue3-April, 2016, pp235-243
http://www.bipublication.com

Research Article

Effect of Different N Fertilization Times on Post-Harvest Quality of Spinach (*Spinacea oleracea* L.)

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ABSTRACT
The present study was conducted to evaluate the effects of urea fertilization times on postharvest quality of spinach. For this purpose, a pot factorial experiment in the context of randomized complete block design with four replications was carried out. Treatments include three fertilization times (25, 50 and 75 days after sowing) and measurements of chlorophyll, vitamin C and, carbohydrate content as well as, weight loss and electrolyte leakage were performed on 0, 7 and 14 days after harvest. Results showed that urea fertilization on 25 days after sowing has the highest effect on post-harvest quality. Significant chlorophyll reduction was observed after 14 days of storage. The highest chlorophyll content at harvest and fertilization times was observed 25 days after sowing. Nutritional value (measured as vitamin C content) and market quality (chlorophyll content) were highest at harvest time and decreased during storage period. Total vitamin C significantly decreased after 14 days at the third times of fertilization (75 days after sowing). Carbohydrate content, weight loss and electrolyte leakage significantly decreased after 14 days of storage; so that the highest rate of carbohydrate and electrolyte leakage was observed at 14 days after the harvest and urea fertilization time as 75 days after sowing.

Keywords: fertilization, post-harvest, nitrogen, *Spinacea oleracea*, quality.

1. INTRODUCTION
Fertilization represents an important and even necessary practice that is widely used by farmers and vegetable growers to compensate soil nutrient loss and enhance crop yield (Rettke et al., 2006; Chintala et al., 2012). Nitrogen is one of the principal nutrients which needed for crops and hence, its deficit in soil has negative consequences on growth and development of crops. Therefore, fertilization with N is a popular horticultural practice extensively applied all around the world (Anjana, 2007). In fact, nitrogen is the most commonly applied nutrient in orchard crops and normally applied at higher rates than most other nutrients (Wells, 2013). However, application of N fertilizers, especially in the horticultural crops, has been always accompanied by some concerns. Apart from economic problems (Huett & Diroy, 2000), it is well documented that excessive use of fertilizers is associated with soil and water contamination, increased rate of pest and disease occurrence, and human health problems caused by over-accumulation of nitrogen in crop tissues (Cantwell & Kasmire, 2003; Chintala et al., 2013 & 2014). Indeed, a harmful ecological problem caused by accelerated agriculture is run-off from croplands which leads to deterioration of water quality and declining sea-life. Therefore, it is of

great importance to implement an efficient nitrogen management system that determines the optimal N fertilizer rate applied in the farms (Pahlmann et al., 2016; Chintala et al., 2013). Considering this paradox, a major challenge for the researches and producers is to sustain global food security and, at the same time, to minimize the negative impact of N fertilizers on the environment.

Another aspect of fertilization that possesses great importance in horticulture is its potential contribution in postharvest characteristics of vegetables and other freshly used crops. Indeed, it has been reported that fertilizers may affect vegetable appearance, texture and taste (Havlin et al., 2005). Postharvest properties of horticultural crops which control storage potential have been also reported to be affected by fertilization because they affect crop susceptibility to mechanical damage, physiological disorders, and decay (Wang, 2003).

Spinach (*Spinacea oleracea* L.) is one of the most important leafy vegetables from *Chenopodiaceae* family which represents an important source of minerals. It is a leafy cool season vegetable that produces a rosette during the vegetative stage (Kansal et al., 1981). Leafy vegetables are important part in the human diet that are used as a main source of vitamins, minerals and other bioactive compounds (Anjana et al., 2007). Spinach has low calorie and contains considerable amounts of vitamins A, C and minerals especially iron.

However, it is a highly susceptible vegetable and various postharvest conditions can affect its quality and marketability (Hodges & Forney 2003). Spinach is a leafy crops and therefore, its marketability highly depends on freshness and turgidity of its leaves. However, many properties of spinach which is making it acceptable by customers are lost quickly during postharvest practices (Grozeff et al., 2010). The loss of turgidity, carbohydrates, chlorophyll and antioxidants such as vitamin C are among the characteristic changes taking place during postharvest periods (Ferrante et al., 2007). Therefore, it has been a major challenge for farmers and horticulturists to postpone these senescence-related traits to extent spinach availability in marketplace.

Regarding to the importance of postharvest traits of spinach leaves in marketability of the vegetable and considering the effects of N fertilizers on physiological properties, texture and appearance of leafy crops, the present study was carried out to investigate the influence of various N fertilization times on postharvest properties of spinach. To get a closer vision on the effect of N fertilization times on postharvest traits, four indices including electrolyte leakage, chlorophyll, carbohydrates and vitamin C content were evaluated under the fertilization regimes.

2. MATERIALS AND METHODS

2.1 Plant Material and Sample Preparation

The experiment was carried out in the research greenhouse of Ferdowsi University of Mashhad as a pot factorial experiment in the context of randomized complete block design with four replications in 2014. Mean minimum and maximum temperatures in the greenhouse were 15 and 26 °C, for the winter crop.

The treatments included urea fertilization times (25, 50, 75 days after sowing; at the rate of 200 kg/ha which is equal to 0.2 g soil (topsoil farm) per pot, and post-harvest times (0, 7, 14 days after harvest). The harvest time was determined when the control parcel’s plants reached to 4-5. leaves per plant and the harvest were done by hand.

2.2 Processing, Packaging and Storage Conditions

100 days after sowing, plants were transferred from greenhouse to the laboratory. Diseased plants were removed; leaves were washed; packed in polyethylene perforated bags and then transferred to the refrigerator with 95% humidity and a temperature of 7 °C. Sampling and re-

measurements were carried out on 0, 7 and 14 days.

2.3 Electrolyte Leakage Determination

Electrical conductivity measurements were performed as described by Blum and Ebercon (1981). The electrolyte leakage was calculated by the following equation.

$EL = (Ci/Cs)\times100$

2.4 Total Carbohydrate Determination

3 ml of anthron indicator was added to the prepared samples at this stage and then they were boiled at 100 °C for 60 minutes (Hedge and Hofreiter, 1962).

2.5 Chlorophyll Content Determination

The total chlorophyll content of the leaves was determined by the method of Arnon (1949). based on acetone extraction of the chlorophyll and colorimetric assay with the aid of a Spectrophotometer Model JENWAY 6305.

2.6 Vitamin C Determination

1 g of fresh plant tissue samples were dissolved in 3 ml of 1% metaphosphoric acid solution, and samples were centrifuged for 15 minutes at 600 rpm. 0.5ml of dichlorophenol indophenol was added to each sample and absorbance rate was read at 500 nm (Institute of Standards and Industrial Research of Iran No. 56098).

2.7 Statistical Analysis

The statistical analysis of the data was performed using SPSS16.0 software for mean comparison based on least significant difference (LSD) test ($p<0.05$). Graphs were drawn by Excel software.

3. RESULTS AND DISCUSSION

According to the results of this study, significant differences were observed between the electrolyte leakage records during storage (Figure 1). These differences were more apparent at the end of the storage period where the electrolyte leakage increased twice as much for samples, compared with the initial values.

The highest electrolyte leakage was observed at day 14 and fertilizing 25 days after sowing (Table 1, Table 2). Electrolyte leakage was the lowest in spinach exposed to late fertilization notably at the end of the storage period (Table 1).

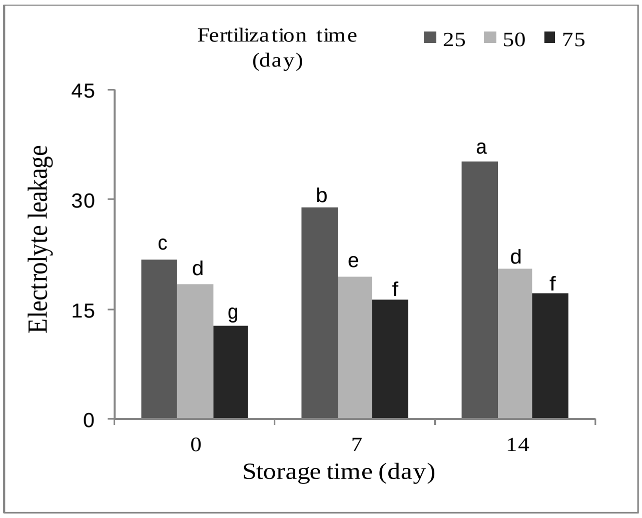

Figure 1. Electrolyte leakage in spinach at harvest and after storage in relation to the urea fertilization time.Means followed by the same letter do not differ by LSD test at $p\leq 0.01$.

Electrolyte leakage has been frequently used as an indicator for tissue and membrane integrity in postharvest quality studies. Previous researches have been shown that it had close correlation with product quality and shelf life (Kou et al., 2012). The use of electrolyte leakage is an effective way to estimate the effect of membrane destructive processes in plant tissues under the influence of environmental factors (Azizpour et al., 2010). The causes of membrane disruption are unknown. However, decrease in cell volume increases the viscosity of the cytoplasmic components which consequently leads to increased probability of molecular interactions and protein denaturation and membrane fusion, and thus the first part of the damaged cell, the loss of membrane integrity results in an increase in electrolyte leakage (Folkert et al., 2001). An increased rate of electrolyte leakage has been used as an indicator of physical damage to cell membranes during storage of tomato fruit. Results indicate that electrolyte leakage in mature-green tomatoes increased with chilling independently of ripening during long term storage (Biswas et al., 2011).

Table 1. The mean values of influence urea fertilization time on quality of spinach

Fertilization time (day)	Chlorophyll a	Chlorophyll b	Total chlorophyll	Weight loss (g)	Electrolyte leakage	Carbohydrate (µg ml $^{-1}$)	Vitamin C (mg ml $^{-1}$)
25	3.31 ^{a}	1.22 ^{a}	4.60 ^{a}	12.59 ^{a}	37.98 ^{a}	0.079 ^{a}	0.37 ^{a}
50	3.28 ^{a}	1.26 ^{a}	4.53 ^{a}	9.91 ^{b}	24.03 ^{b}	0.080 ^{a}	0.34 ^{b}
75	3.13 ^{a}	1.20 ^{a}	4.33 ^{a}	9.80 ^{b}	16.98 ^{c}	0.079 ^{a}	0.37 ^{a}

Means that differ significantly within columns are followed by differ letters by LSD test at $p\leq 0.01$.

In the present experiment, loss of the chlorophyll content was observed in spinach. The lowest chlorophyll content was observed on 14 days after harvesting and the last fertilization time (Figure 2, Table 2). Since greenness is an important trait in leafy vegetables, reduction of chlorophyll concentration lowers marketability of spinach. On the other hand, the highest chlorophyll content was obtained in the first urea fertilization time (on the 25th day) indicating that this fertilization time is ideal for retention of chlorophyll content and leaf greenness (Table 1). This finding is in accordance with the results reported by Gutierrez-Rodriguez (2012) who showed that post-harvest nutritional quality and shelf life of spinach is significantly reduced when cultivated at high rate of nitrogen concentration.

Table 2. The mean values of influence storage time on quality of spinach

Storage time (day)	Chlorophyll a	Chlorophyll b	Total chlorophyll	Weight loss (g)	Electrolyte leakage	Carbohydrate (µg ml $^{-1}$)	Vitamin C (mg ml $^{-1}$)
0	3.48 ^{a}	1.59 ^{a}	5.70 ^{a}	12.18 ^{a}	15.24 ^{c}	0.75 ^{b}	0.70 ^{a}
7	3.28 ^{a}	1.05 ^{b}	4.50 ^{b}	10.41 ^{b}	19.49 ^{b}	0.80 ^{a}	0.22 ^{b}
14	2.96 ^{b}	1.04 ^{b}	4.00 ^{c}	9.35 ^{c}	30.26 ^{a}	0.82 ^{a}	0.12 ^{c}

Means that differ significantly within columns are followed by differ letters by LSD test at $p\leq 0.01$.

The loss of chlorophyll at both storage temperatures was not affected by N application rates between 140 and 260 mg l^{-1}, thereby improving the retention of visual quality, as also indicated for broccoli inflorescences (Dan et al., 2005). Among various aspects of tissue decay, color is a very important determinant that influences consumer choice (Ferrante and Maggiore, 2007). Greenness of leafy vegetables is due to chlorophyll pigments that quickly undergo degradation during postharvest and storage period (Nisha et al., 2004).

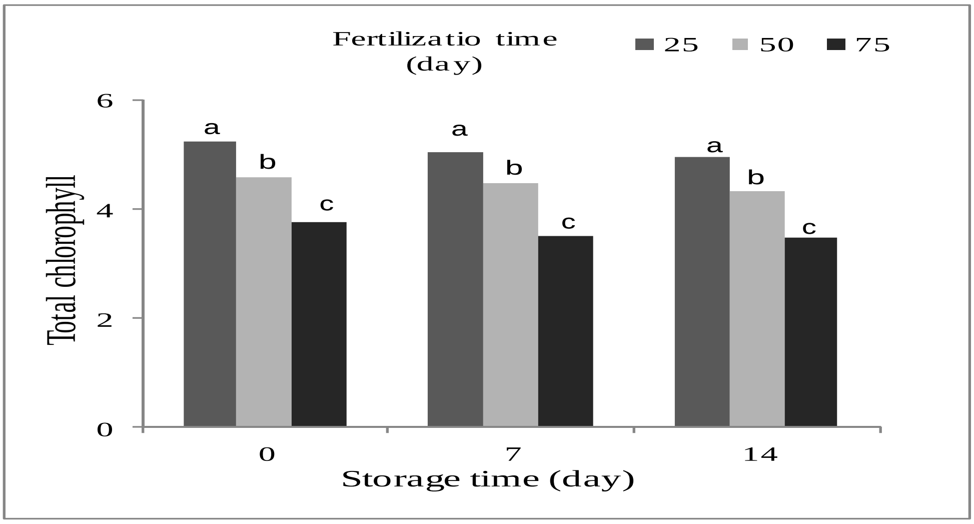

Figure 2. The concentration of total chlorophyll in spinach at harvest and after storage in relation to the urea fertilization time.Means followed by the same letter do not differ by LSD test at $p\leq 0.01$.

Leaf discoloration is attributed to degradation of the leaf pigments or tissue browning. These physiological processes occur when cell membranes lose their integrity and release enzymes, which come into contact with their substrates, generating postharvest disorders (Jacxsens et al., 2002). Internal qualities such as antioxidant components are also important for human health because of their nutritional value. Chlorophyll and carotenoids are tightly correlated with each other because the latter protects the chlorophyll from photo-oxidation during growth (Biswall, 1995). This phenomenon has been observed in many leafy vegetables. It is known that the chlorophyll content of leafy plants such as chicory, Swiss chard and valeriana lettuce (Ferrante and Maggiore, 2007) decreases during storage.

The vitamin C concentration was highest at harvest time and decreased with urea fertilization close to harvest time (Table 1, Table 2). Moreover, vitamin C concentration declined during storage, so that the lowest content of vitamin C was observed at 14 days after harvesting and the last urea fertilization time (Figure 2). Vitamin C content was reduced as storage duration increased. This can be attributed to the structure of ascorbic acid that is gradually oxidized under poor storage condition, and hence, results in reduction of nutritional value of the crop (Chumillas et al., 2007). Vitamin C is a critical part of the plant defense systems (Hodges and Forney, 2003). It has been reported that higher ascorbic acid content in spinach is associated with better visual quality during storage (Bergquist et al., 2006). Vitamins are a class of organic compounds required in the human diet for normal physiological functions; and their unavailability in the diet causes vitamin deficiency diseases. Leafy crops, especially when consumed fresh, provide a valuable source of vitamin C for the human diet. Ascorbic acid is sensitive to destruction when fruits and vegetables are subjected to adverse postharvest handling and storage conditions (Lee and Kader, 2000). The vitamin is vulnerable to chemical and enzymatic oxidation, highly water soluble, and is a sensitive and suitable marker for monitoring quality change during postharvest practices (Morrison, 1974).

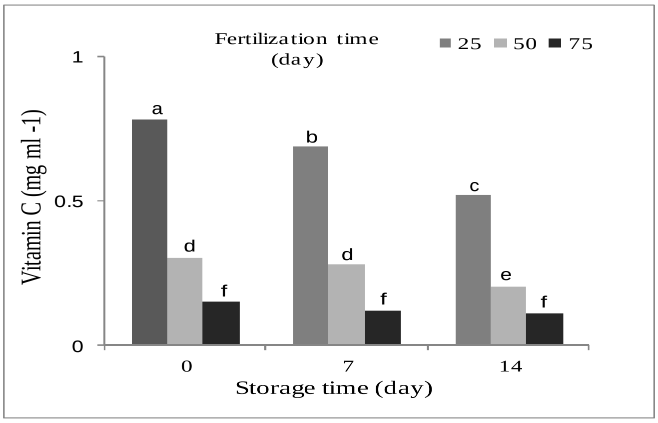

Figure 3. The concentration of vitamin C in spinach at harvest and after storage in relation to the urea fertilization time. Means followed by the same letter do not differ by LSD test at $p \leq 0.01$.

Konstantopoulou (2010) reported reduction of chlorophyll and vitamin C content during 10 days of storage at 5 or 10 °C. Hirata et al. (1987) also found that in leafy vegetables such as pak-choi (*Brassica chinensis* L.), edible amaranth (*Amaranthus mangostanus* L.), and soup celery (*Apium graveolens* L. var. secalinum Aleff.), ascorbic acid content is gradually diminished during storage period. Vitamin C concentration of spinach increases in the range of N (Bergquist et al., 2007). Fujiwara et al. (2005) reported that in spinach, vitamin C concentration increases during the winter season and this increases can reach up to 7-fold when compared to the summer season, being characterized by lower level of vitamin C.

Total carbohydrate is regarded as a good index of the overall quality and shelf life of spinach at harvest and during storage. In this study, the amount of carbohydrates during the storage increased (Table 2). Moreover, this finding may result from reducing water content of spinach leaves, which increased carbohydrate concentration. Carbohydrates are an important flavor component in many vegetables, producing a distinct sweet taste. The balance between storage and soluble carbohydrates within a tissue can affect vegetable quality. Starch is a major source of carbohydrate that, when degraded, contributes to sweetening of certain vegetables. Conversely, biosynthetic reactions can detract from sweetness. Starch metabolism during postharvest handling can have a significant effect on vegetable quality. In most harvested crops, decreased starch content results in increased sweetness. In leafy vegetables with a strong dynamic capacity to interconvert starch and soluble sugars, over- or under-accumulation of starch is rarely a quality problem (Schaffer and Petreikov, 1997). Few studies have been conducted on carbohydrates content of vegetables during storage. The increase in total sugar content can be attributed to conversion of starch into sugars (Tsuda et al., 1999).

The results presented in this paper demonstrated that the pre-harvest treatment of spinach concerning fertilization times improves the post-harvest storage quality. Fertilization after 25 days of spinach caused the highest shelf life and the lowest weight loss after harvest (Table 1). Fertilization times were applied as root irrigation which resulted in an enhanced storage quality (Table 2, Figure 4). This suggests that fertilization times might have induced certain systemic physiological changes in the plant and led to improved post-harvest quality and shelf life of spinach leaves. Spinach is an important leafy vegetable regarding its nutritional quality, and ranks third in total antioxidant capacity, behind garlic (*Allium sativum*) and kale (*Brassica oleracea*) (Bunea et al., 2008). The genotype and growth conditions impact plant metabolism, which in turn can affect crop quality at harvest and post-harvest storage (Weston and Barth, 1997). Commercially, spinach leaves are stored in polypropylene bags at low temperatures close to 0 °C (Bergquist et al., 2006) but often they are kept in the range of 4–10 °C. Post-harvest changes in spinach may include losses in visual quality and nutritional value (Conte et al., 2008). Nitrogen availability has a significant effect on the nutritional quality of spinach. Despite this well-known effect, the specific influence of postharvest quality is poorly understood (Gutierrez-Rodriguez, 2012).

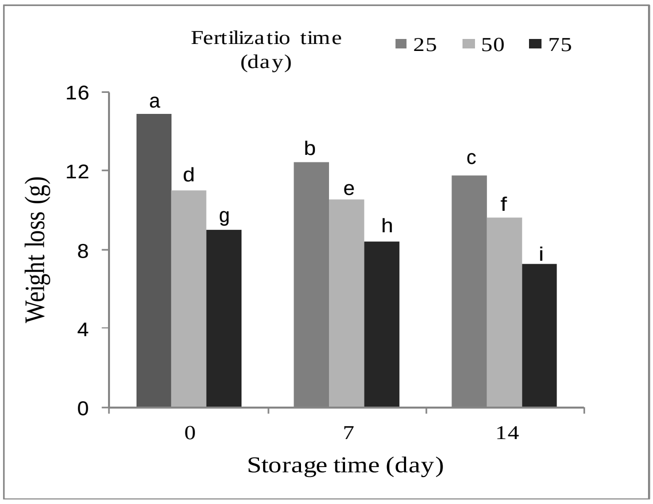

Figure 4. Weight losing spinach at harvest and after storage in relation to the urea fertilization time. Means followed by the same letter do not differ by LSD test at $p \leq 0.01$.

In general, the results obtained in the present study indicated that application of appropriate urea fertilization can contribute to improvement of postharvest quality of spinach; a vegetable often sold as fresh cut leaves in market place. Our findings provide the evidence supporting the idea that applying an optimal N fertilizer rate provides sufficient nitrogen for normal growth and desired post-harvest properties of the crop and, at the same time, avoid negative consequences of excess nitrogen availability in the soil. Based on these results, fertilizing the soil 25 days after sowing seem to be the best fertilization time for achieving a yield of high post-harvest quality and marketability. A suitable fertilization practice at right time may have a positive effect on marketability of spinach and perhaps other leafy vegetables.

ACKNOWLEDGEMEN:
This study was supported by the Department of Gardening Faculty of Agriculture Ferdowsi University of Mashhad (FUM).

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