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Evaluation of Nitrate and Nitrite Content of Iran Southern (Ahwaz) Vegetables During Winter and Spring of 2006

Ahmad Shahlaei, Naser Alemzadeh Ansari and Farideh Sedighie Dehkordie
Department of Horticulture, Faculty of Agriculture, Shahid Chamran University, Ahwaz, Iran

Abstract: Vegetable crops have main role in healthy and diet of people. High nitrate and nitrite concentration then limited value of vegetable crops that have threat for human health. From winter to middle spring, leafy vegetables are produced for many markets of Iran in Khuzestan province. To evaluation of nitrate and nitrite content, sampling of vegetables was conducted in Ahwaz's (center of Khuzestan province) markets and fields in winter and spring of 2006. The results showed that there were significant differences ($p<0.01$) in nitrate content between various vegetables. Also, there were significant differences ($p<0.01$) in nitrite content of various vegetables and seasons of sampling. The effect of seasons on nitrate content was not significant. Celery petioles (3015.8 mg kg^{-1} Fresh Weight (FW)) and potato (184.4 mg kg^{-1} FW) have the highest and lowest nitrate content between all kinds of vegetables, respectively. The highest and lowest nitrite content were for tomato (9.45 mg kg^{-1} FW) and mint (1.51 mg kg^{-1} FW), respectively. The mean nitrate content of fruit bearing, leafy and bulbous and tuberous vegetables were 999.3, 862 and 666 mg kg^{-1} FW, respectively. These values for nitrite were 6.8, 2.96 and 3.63 mg kg^{-1} FW, respectively. In the almost of the fruit bearing vegetables (such as tomato and cucumber) and tuberous ones (such as potato and carrot) the nitrate content was higher than limited values. While leafy vegetables nitrate content was lower than allowable levels.

Key words: Nitrate, nitrite, vegetables quality, human health, allowable levels

INTRODUCTION

Vegetables are calculated a port of main foods for world population especially for developed countries; because by changing of the lifestyle and working, cause to decrease of body movement, so using of food that has low energy and high nutrition value is needed (Rubatzky and Yamaguchi, 1997). Vegetables have high nutrition value, of vitamins, minerals and biologically active compounds (Kmiecik *et al*., 2004).

The knowledge of correct nutrition that is base on biological value and food quality is needed for the health of the human body. Many of plants products when consumes in high quantity have harmful effects. Nitrate and nitrite are detrimental components in plants after applying of nitrogen fertilizers in soil and oxidation by microorganism, the produced nitrate have high affinity to absorption by plants (Anonymous, 1992; Hogg *et al*., 1992; McKnight *et al*., 1997). Huarte-Mendicoa *et al*. (1997) believed that most of the total nitrate content in a normal diet is a direct result of vegetable intake.

The amount of nitrate content is one of the most important factors of vegetables quality. European Union Food Commission (CECSCF, 1992) states the daily acceptable intake level of nitrate and nitrite as 0-3.65 and 0-0.07 mg kg^{-1}, respectively. Similarly, FAO and WHO food commission report the average daily nitrate and nitrite intake of a 60 kg person as 220-240 and 16-32 mg, respectively.

Excessive amount of nitrate in food causes the following problems in human's body: Young babies with low stomach acidity may suffer from infantile methemoglobinemia due to excessive nitrates in their diet, where nitrate is substituted the oxygen in hemoglobin and death may occur (Ezeagu, 1996; Bruning-Fann and Kaneene, 1993a) and various cancer (Tannenbaum and Correa, 1985; Huarte-Mendicoa *et al*., 1997). The lethal dose of nitrite for an adult ranges from 30 to 250 mg kg^{-1} and the average acceptable daily dose ranges from 0.4 mg kg^{-1} (FAO/WHO study) to 13 mg kg^{-1} (Corre and Breimer, 1979).

Tabatabaei (2005) determined the levels of nitrate in tuberous, leafy and fruit bearing vegetables of Tabriz of Iran. They showed that the amount of leafy vegetables nitrate was higher than tuberous vegetables and these ones were higher than fruit bearing vegetables. Also, it was shown that the nitrate content of leafy vegetables was high; for example in cress and sweet fennel was

Corresponding Author: Ahmad Shahlaei, Department of Horticulture, Faculty of Agriculture, Shahid Chamran University, Ahwaz, Iran

4230 mg kg^{-1} FW. Overall, the mean nitrate content of leafy vegetables was 2020 mg kg^{-1} FW. These values for fruit bearing and tuberous vegetables were 770 and 158 mg kg^{-1} FW, respectively.

The nitrate content of vegetables based on dry weight was determined (Malakouti, 2002). They stated that not only the nitrate content of various vegetables is not same but also different parts of same vegetables have different nitrate content. Totally, turnip and spinach's petioles had highest nitrate content and tomato had lowest nitrate content.

The nitrate content of vegetables, fruits and drinking water in summer and winter seasons of Belgium was determined (Dejonckheere and Steurbaut, 1994). They showed that fruits nitrate content was lower than vegetables nitrate. Among all kinds of studied vegetables, leafy vegetables nitrate like lettuce and spinach was higher than other vegetables. Vegetables grown in the fall at high latitudes in the presence of nitrate fertilizer, low air temperatures, frost events and low light intensity, will generally have increased levels of nitrates. In a greenhouse study, nitrate levels in vegetables grown under glass increased from October to January (Stopes *et al*., 1988). A similar study by Santamaria *et al*. (1999) measured nitrate concentrations ranging from 1622 ppm in spring grown spinach to 2580 ppm in a fall/winter grown crop. Nitrate levels typically decline after harvest-with the rate of decline related to storage temperatures and initial nitrate levels (Aworh *et al*., 1980).

The United Kingdom submitted the results of numerous monitoring surveys for nitrate in lettuce and spinach and in other vegetables. The nitrate concentrations in lettuce collected over all seasons for a number of years ranged between 50 and 5300 mg kg^{-1}, while those in spinach were between 25 and 4600 mg kg^{-1}. The concentration in other vegetables were <3 mg kg^{-1} for Leeks and 4200 mg kg^{-1} for radishes (Muramoto, 1999)

There was a positive relationship between amount of nitrogen fertilizer and level of nitrate and nitrite accumulation in edible parts of lettuce. Nitrate content of outer leaves was higher than inner leaves, so those nitrate and nitrite amounts were lower in evening in comparison to morning harvesting. There had significant difference in nitrate content between cultivar and nitrite accumulation as in Pich Varamini was higher than Pich Ahwazi (Boroujerdnia *et al*., 2007).

Khuzestan province has subtropical weather; autumn and winter are temperate but by the beginning of spring the weather becomes warmer so that at the end of spring becomes over 40°C, many leafy vegetables especially cold season vegetables were grown at autumn and winter. These crops produced for Iran markets. The aim of this research is to establish nitrate and nitrite levels of Ahwaz's vegetables at winter and spring seasons and whether these levels are higher than maximum permissible limit or not.

MATERIALS AND METHODS

All vegetables were bought from Ahwaz's markets and its fields and then they were transited to physiology lab of Horticulture department of Shahid Chamran University of Ahwaz at middle winter and middle spring 2006. After gathering, vegetables were carried to laboratory. The injured parts first were eliminated and then vegetables were divided to small sections and held in acetic acid 2% for 1 min and rinsed in distill water. After 48 to 72 h holding in 70°C oven, dried vegetables were ground, mashed and held in 4°C until experiment. Nitrate and nitrite content of samples was measured by Di-AZO and spectrophotometer method. Statistical analysis as factorial based on completely randomized design was accomplished using the SPSS software package and the means were separated by Duncan's multiple range test or t-test.

RESULTS

Vegetables nitrate: The results are shown that there were significant differences among nitrate content of various vegetables at $p<0.01$. Among all kinds of vegetables, potato with 184.4 and celery petioles with 3105.8 mg kg^{-1} Fresh Weight (FW) had the minimum and maximum nitrate content respectively (Fig. 1).

The vegetables considered in the present study based on nitrate content can be divided into four groups: First group: Vegetables containing low nitrate levels (100-500 mg kg^{-1} FW): potato, mint, garlic. Second group: Vegetables containing medium nitrate levels (500-1000 mg kg^{-1} FW): Cauliflower, radish leaves, sweet fennel, turnip, carrot, parsley, coriander, kurrat, cabbage, cress, sweet pepper, eggplant, onion, lettuce inner leaves, spinach, cucumber, basil. Third group: Vegetables containing high nitrate levels: (1000-1500 mg kg^{-1} FW) squash, root beet, celery leaves, radish root (tuber) and tomato. Fourth group: Vegetables containing very high nitrate levels (1500-3000 mg kg^{-1} FW): lettuce outer leaves, celery petioles (Table 1).

Although differences between nitrate contents of cold and warm seasons vegetables were not significant, but cold season vegetables nitrate content was higher than warm season while diffrences between nitrite content of cold and warm seasons vegetables were significant as way cold season vegetables nitrite content was higher

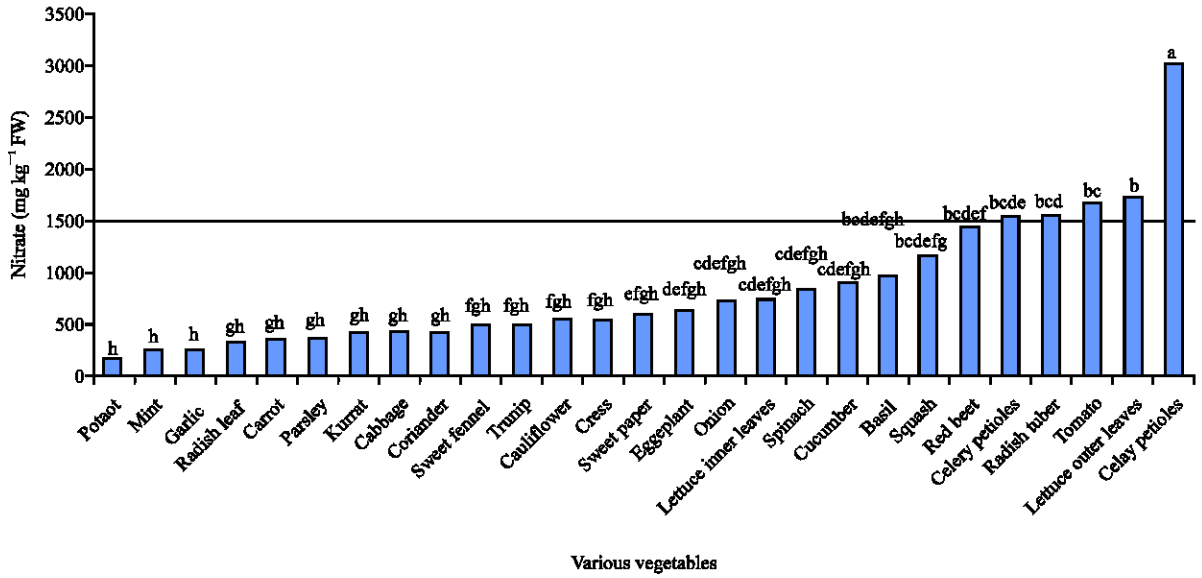

Fig. 1: Average nitrate content of various vegetables in winter and spring seasons (mg kg^{-1} FW) (Means followed by the same letters did not differ significantly at 5% levels of probability)

Table 1: Maximum, minimum and average nitrate content (ppm in kg^{-1} FW) of various vegetables of Ahwaz at winter and spring of 2006 and maximum tolerated nitrate concentrations

	Winter				Spring			
Type of vegetables	Max	Min	Average	Allowable limit*	Max	Min	Average	Allowable limit*
Cabbage	859.5	28.0	428.3	900	1014	21.0	503.8	500
Lettuce inner leaves	911.0	300.0	583.1	4000	1522	116.0	862.0	3000
Lettuce outer leaves	2866.0	483.0	1705.0	N	3172	283.0	1739.0	N
Spinach	1400.0	422.0	886.5	4500	1217	483.0	748.0	3500
Radish leaves	483.0	55.0	179.0	N	911	178.0	495.6	N
Radish tuber	1950.0	608.0	1205.0	1400	5617	608.0	1902.0	1400
Cress	1706.0	55.0	678.6	N	911	56.0	434.2	N
Sweet fennel	1583.0	177.5	654.1	N	544	488.0	313.3	N
Basil	1460.0	544.0	996.2	N	1889	438.0	948.8	N
Mint	361.0	55.0	154.4	N	972	422.0	349.6	N
Coriander	910.0	488.0	569.4	N	910	116.0	310.1	N
Parsley	1277.0	100.0	358.6	N	1094	116.0	374.0	N
Carrot	544.0	238.0	251.9	1500	1217	55.0	458.3	1500
Tomato	2500.0	544.0	1644.0	300	2500	544.0	1681.0	300
Cucumber	2317.0	361.0	999.3	150	1828	177.5	813.3	150
Sweet pepper	850.0	300.0	574.5	N	1522	361.0	622.5	N
Potato	360.0	55.0	213.8	60	360	299.0	155.0	60
Kurrat	1094.0	228.0	445.8	N	605	55.0	385.2	N
Celery leaves	5127.0	850.0	2169.0	5000	1704	605.0	887.4	5000
Celery petioles	5829.0	1345.0	3827.0	4000	4293	363.0	2205.0	4000
Onion	2561.0	667.0	1263.0	1000	577	300.0	248.4	1000
Squash	1460.0	100.0	845.3	N	2805	483.0	1471.0	N
Eggplant	1399.0	421.0	864.5	N	1399	116.0	652.4	N
Garlic	483.0	55.0	197.7	N	725	55.0	229.3	N
Turnip	1197.0	98.0	501.3	N	-	-	-	N
Red beet	1972.0	1142.0	1429.0	4000	-	-	-	N
Cauliflower	1032.0	100.0	554.9	N	-	-	-	N
Average	1647.8	363.6	895.5		1637.8	280.7	782.8	

Max: Maximum, Min: Minimum, -: These vegetable species was not found in Ahwaz markets and fields at spring season and N: Data do not find. *- Data Sohn and Yoneyama (1996) and Maff (1999)

than warm season. Some of nitrate contents of cold season vegetables is lowest in winter than to spring but nitrate content of warm season vegetables is lowest in spring than to winter. For example cold season vegetables are cabbage, lettuce, radish, carrot and warm season vegetables are basil and cucumber (Table 1, 2). This

Table 2: Maximum, minimum and average nitrite content (ppm) of various vegetables of Ahwaz at winter and spring of 2006

Type of vegetables	Winter			Spring		
	Max	Min	Average	Max	Min	Average
Cabbage	8.1	2.30	5.225	5.2	1.90	3.200
Lettuce inner leaves	10.3	1.60	4.360	7.6	1.40	4.000
Lettuce outer leaves	9.3	1.10	4.060	6.2	2.10	3.200
Spinach	7.1	0.68	3.416	4.7	0.72	2.507
Radish leaves	4.5	1.40	2.540	8.2	1.20	3.520
Radish tuber	6.9	2.10	4.560	5.2	2.80	4.200
Cress	4.5	1.10	2.340	7.4	1.10	2.820
Sweet fennel	4.7	1.90	3.100	4.5	1.40	2.020
Basil	5.2	2.30	3.800	4.0	1.10	1.520
Mint	4.0	1.20	1.900	2.1	0.71	1.132
Coriander	3.8	1.20	2.300	2.3	1.10	1.940
Parsley	2.1	0.96	1.592	4.8	1.60	2.880
Carrot	5.2	0.23	2.906	5.2	2.10	3.680
Tomato	12.4	6.20	10.130	12.8	4.70	8.920
Cucumber	9.1	0.28	5.320	7.9	0.28	4.636
Sweet pepper	9.8	3.50	7.375	8.8	4.70	6.525
Potato	2.8	0.00	1.840	2.6	1.40	1.820
Kurrat	4.5	2.10	3.160	6.4	2.30	3.800
Celery leaves	7.1	0.71	3.982	5.2	0.71	3.342
Celery petioles	9.1	0.61	4.182	3.6	0.40	1.636
Onion	9.5	1.40	5.625	9.3	1.40	4.250
Squash	10.0	2.80	6.775	12.1	2.30	6.675
Eggplant	10.0	5.00	7.925	10.0	2.80	5.967
Garlic	3.1	2.40	2.767	5.2	1.20	2.638
Turnip	6.6	0.70	3.275	-	-	-
Red beet	9.0	5.00	6.925	-	-	-
Cauliflower	5.5	3.50	4.800	-	-	-
Average	12.4	0.00	4.162	12.8	0.00	3.635

Max = Maximum, Min = Minimum, - = These vegetable species was not found in Ahwaz markets and fields at spring season

subject did not correct for all vegetables. Vegetables production depends on various factors (temperature, light, humidity, soil type, elements nutrition, cultivar, species, plant age, time of harvesting and interaction between them). Each factor affect on absorption and transformation of nutrient elements. Therefore this phenomenon did not permit one law. But attentions to growth and development physiology of plants can reduce nitrate and nitrite accumulation (Ramachandran *et al*., 2005; Santamaria *et al*., 1999; Boroujerdnia *et al*., 2007).

The vegetables based on edible portions in this survey were divided into 3 groups: first leafy vegetables, second fruit bearing vegetables and third tuberous and bulbous vegetables.

The differences of nitrate content in these 3 groups were significant at $p<0.05$. Bulbous and tuberous vegetables with 666 and fruit bearing ones with 999.33 mg kg^{-1} FW had the lowest and highest nitrate content, respectively. The fact that fruit bearing vegetables nitrate is lower than leafy and tuberous vegetables is a proof but in some cases like our study the nitrate content of fruit bearing vegetables because of factors that increase nitrate content(low light density, low temperature, high nitrogen fertilizers use and etc.) was higher than leafy and tuberous vegetables.

In leafy vegetables, the lowest and highest nitrate content was for mint with 252 and celery petioles with 3015.8 mg kg^{-1} FW, respectively. Based on nitrate content the leafy vegetables can be divided into 3 groups. First group leafy vegetables with low nitrate levels like mint, radish leaves, parsley, Kurrat, cabbage, coriander, sweet fennel, lettuce inner leaves.

Second group: leafy vegetables with medium nitrate levels like spinach, celery leaves, basil. Third group: leafy vegetables with high nitrate levels like lettuce outer leaves, celery petioles. It must be noted that to avoid intake high nitrate levels by human the outer leaves and petioles of these vegetables must be discarded (deleted).

Potato and radish roots (tubers) with 184.4 and 1553.3 mg kg^{-1} FW had the lowest and highest nitrate content in tuberous and bulbous vegetables, respectively.

Generally, we can divide these vegetables based on nitrate content into two groups: 1) low nitrate content like potato, garlic, carrot, turnip and onion 2) high nitrate content like red beet and radish root (tuber).

Among fruit bearing vegetables, highest and lowest nitrate content were for tomato with 1664.7 and Sweet pepper with 598.5 mg kg^{-1} FW, respectively. The differences between nitrate content in eggplant, sweet pepper, cucumber and squash was not significant but there was significant differences at $p<0.05$ in nitrate content between tomato and other fruit bearing vegetables.

Vegetables nitrite: There were significant differences at $p<0.01$ in nitrite content among various vegetables and seasons. Mint with 1.51 and tomato with 9.45 mg kg^{-1} FW had the lowest and highest nitrite content, respectively. If all vegetable on base of their edible portion divided 3 groups. The differences in nitrite content among all kinds of vegetables were significant at $p<0.01$. Highest and lowest nitrite content was for fruit bearing vegetables with 6.8 and leafy vegetables with 2.96 mg kg^{-1} FW, respectively. Mean nitrite comparison of vegetables in Fig. 2.

Among leafy vegetables, mint with 1.51 and lettuce inner leaves with 4.18 mg kg^{-1} FW had the lowest and highest nitrite content, respectively. Highest and lowest nitrite content of bulbous and tuberous vegetables was belonged to red beet with 6.92 and potato with 1.83 mg kg^{-1} FW, respectively. Although there were no significant differences between garlic, turnip and carrot nitrite content with potato.

High differences in nitrite content were shown between fruit bearing vegetables. Among these vegetables, highest and lowest nitrite content was for tomato with 9.45 and cucumber with 4.94 mg kg^{-1} FW, respectively.

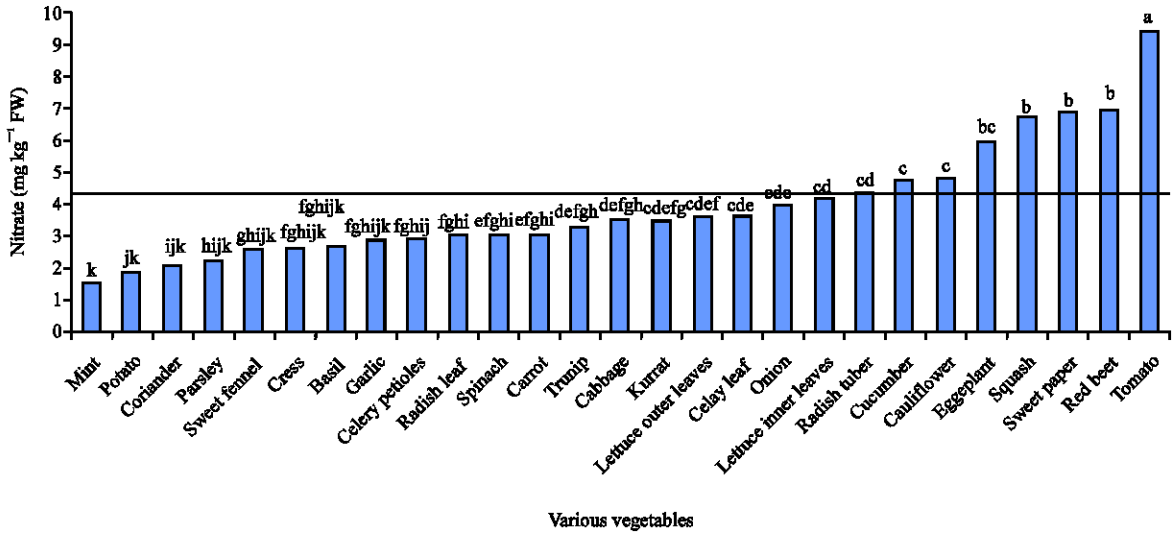

Fig. 2: Average nitrite content of various vegetables in winter and spring seasons (mg kg^{-1} FW) (Means followed by the same letters did not differ significantly at 5% levels of probability)

Average nitrite amount of vegetable crops at winter season (with 4.16 mg kg^{-1} FW) was more than spring season (with 3.63 mg kg^{-1} FW).

DISCUSSION

The results of experiment showed that nitrate amount in various vegetables with together were different. Changes range of nitrate in various vegetables were from 182 in tuber potato to 3105 celery petioles, many factors effect in nitrate concentration of vegetables. The plant genotype determines nitrate uptake from soil and its assimilation into nitrate. Some plants reduce nitrate to nitrite and ammonium ions in roots while others assimilate only a small part of the nitrate in roots and the rest in the above ground plant parts. A third group of plants reduce nitrate to ammonium ions only in the aerial plant parts. The process is affected by different activities of nitratreductase and nitritreductase in the various plants parts, for example, nitrate is transported to the above ground plants in many cereals, root and cruciferous vegetables, grasses, etc. Partial nitrate reduction to ammonium ions occur in plant roots in many Leguminose and Umbelliferous plants, corn, etc. In this (survey) research, great differences between nitrate and nitrite content of various vegetables were observed. It was shown by different studies that various families have different nitrate accumulation (Staugaitis and Dris, 2002).

Vegetables families according to their nitrate levels were separated as follows (Santamaria *et al*., 1999):

Families containing high nitrate levels: Chenopodiaceae, Brasicaceae, Apiaceae and Asteraceae.

Families containing low nitrite levels: Convolvulaceae, Solanaceae and Alliaceae.

The vegetables families analyzed in our study can be listed by decreasing nitrate and nitrite content as follows:

Apiaceae> Asteraceae> Chenopodiaceae>
Solanaceae> Alliaceae> Brasicaceae>
Lamiaceae accoroding to nitrate levels in vegetables.

Solanaceae> Chenopodiaceae> Asteraceae>
Alliacea> Brasicaceae> Apiaceae> Lamiaceae
according to nitrite levels in vegetables.

Generally, nitrate concentrations tend to be higher in stems and petioles than in leaves so the nitrate level of vegetables that their petioles, stems or leaves are consume must be considered. Nitrate accumulation in older organs is high because activity of nitrate reductase in these parts is low. A survey was reported on nitrate and soluble oxalate concentration in retail fresh vegetables based on the consumed edible portions showed that nitrate content differed in the various portions under examination. Indeed, they listed the vegetables organs analyzed by decreasing nitrate content as follows (Santamaria *et al*., 1999).

Petioles> Leaf> Root> Stem>Inflorescence> Tuber> Bulb

The vegetables organs analyzed in the present study can be listed by decreasing nitrate and nitrite content as follows:

Petioles> Fruit> Tuber> Leaf> bulb nitrate

Fruit> Tuber> bulb> Leaf> Petioles nitrite

These results are different with others researchers (Santamaria *et al*., 1999; Stopes *et al*., 1988) they showed general leafy vegetables especially in fall and winter seasons have high levels of nitrate, but in this experiment nitrate amount were not different between winter and spring seasons. Khuzestan region has low permissible limited whether so leafy vegetables almost were grown in field conditions. Environmental factors such as light intensity, air temperature, soil temperature, fertility and moisture also affect on nitrate levels in vegetables (Ramachandran *et al*., 2005). Temperature is one the most factors. In Khuzestan condition air temperature is suitable for growth and development of leafy vegetables especially for cold season vegetable at winter season but soil conditions cause to limit nitrogen absorption. Also intensive light is another factor cause nitrate decrease in vegetables. the number of cloudy days in the Khuzestan province is very low (sunny hours are 263.1 on a growing month), thus high light intensity causes increasing nitrate assimilation and thereby decreasing nitrate of plant. In evaluating nitrate and nitrite, our results are agreement with Boroujerdnia *et al.* (2007). They showed in Khuzestan province, in production of lettuce in all treatments, nitrate and nitrite content were lower than permissible level. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established Acceptable Daily Intakes (ADI) of nitrate and nitrite as 0-3.7 NO_3 mg kg^{-1} body weight and 0-0.07 NO_2 mg kg^{-1} body weight, respectively (JECFA, 1995). In 1997, the European Union established the maximum levels for nitrate content in lettuce. Hence, it should be advised to protecting quality and quantity leafy vegetable use nitrogen fertilizer proportional with best yield crop and for preventing undesirable. But other vegetables (fruit bearing, bulbous and tuberous) mostly do not produce in Khuzestan province at winter and early spring.

CONCLUSIONS

- Nitrate content of Ahwaz's leafy vegetables was lower than allowable levels, so they were harmless for consumption.
- There was no significant difference between nitrate content of different seasons while nitrite content of winter was higher than spring ones.
- In spite of the fact that nitrate content of different vegetables like Potato, Onion and fruit bearing ones which have daily consumption is higher than limited levels, so we need some programs to avoid nitrate accumulation in these crops.

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