

Assessing the Health Risk of Nitrate Content in Vegetables to the General Population in Tehran-Iran

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Abstract

Nitrate is found naturally in foods and in high concentrations in certain vegetables. European Union Food Commission (CECSCF, 1992) states the daily acceptable intake level of nitrate as 0-3.65 mg/kg. The objective of this study is to assess the relative safety of Tehran leafy vegetables based on world standard nitrate limits & potential health risk to local inhabitants. A systematic survey of nitrate and nitrite concentrations in vegetables from 910 samples (from 10 different markets during 12 consecutive months of 2011) in Tehran was carried out. Results were compared with the permissible limits (PL), acceptable daily intake (ADI) and provisional maximum tolerable daily intake (PMTDI) as set by World Health Organization (WHO), Food and Drug Administration (FDA) and Joint FAO/WHO expert committee on food additives (JECFA). The results indicated that the mg of nitrate concentrations/kg fresh weight in vegetables ranged from 820 to 4788 in lettuce, 3004 to 5011 in celery and 2989-5036 in spinach, with average concentration of 2989-5036 mg/kg FW, respectively. The estimated daily intake (EDI) of nitrate from lettuce and cabbage showed nitrate levels were much higher than maximum EC levels. Potential health risks from exposure to nitrate in these vegetables need more attention. A highly significant, although low, positive correlation was found between nitrate and nitrite contents of the winter and spring grown vegetables, compared to summer and autumn grown vegetables.

Keywords: Nitrate; Nitrite; leafy vegetables; Risk assessment; Tehran

Introduction

Nitrate content is an important quality characteristic of vegetables. Vegetable nitrate content is of interest to governments and regulators owing to the possible implications for health and to check that controls on the content are effective. Nitrate itself is relatively non-toxic but its metabolites may produce a number of health effects [1]. More than three quarters of our average nitrate intake comes from vegetables [2], which provide about 80% of the average daily dietary intake [3]. Vegetables that may accumulate nitrate in their tissues are leafy vegetables such as Spinach, Lettuce, and Cabbage, or root crops like carrot, Potatoes, and others like Cauliflower, beans and peas. The Acceptable Daily Intake (ADI) of nitrate and nitrite set by European Commission's Scientific Committee for Food (ECSCF), is 3.7 mg/kg body weight, and 0.06 mg/kg body weight, respectively [WHO, 1995] [4]. The nitrite and nitrate concentrations in vegetables depends on a number of factors such as season, light, temperature, method of growth, species variation and fertilizers. Agriculture is considered the major source of nitrate and nitrite in the environment [5]. The excessive use of the pesticides and fertilizers in agriculture with the threat of these chemicals in crops and water has become one of the most important public awareness issues. Therefore, the aim of this work was to examine some of the most common vegetables found in Tehran markets for its nitrate and nitrite contents.

The Objectives of the Present Work

- Determination of the level of nitrate and nitrite in Lettuce, Celery, Spinach, Cabbage, Chinese cabbage, carrot and potato crops sold in Tehran markets.
- Determination the effect of seasons on the level of nitrate and nitrite in Tehran's leafy vegetable and crops sold in Tehran markets.
- Assess the associated health risk posed to the Tehran population through exposure to nitrate from vegetables.

Materials and Methods

Sample collection

910 samples (from 10 different markets during 12 consecutive months of 2011) in Tehran were purchased. Crops were collected for this study: Potato, Carrot, Iceberg and Romania Lettuce, Celery, Spinach, and Chinese cabbage crops. These are the most common purchased vegetables in Tehran markets. Sampling was replicated twice within each season at intervals of two weeks. To evaluate variability of nitrate content within sub-samples, five plants or bundles of sub-samples were analyzed separately and averaged to produce one sample data point.

Nitrate and nitrite Extraction

A fifty gram sample of the prepared crop was blended with 50 mL distilled water in a home blender. The mixture was filtered through Whatman No.2 filter paper, and the filtrate was passed through a glass column fitted with a tape and filled with activated alumina, in order to separate the green color (Chlorophyll) and get a transparent solution. Water was used as eluting solvent. The eluted solution filtered using 0.45 µm filter paper in order to eliminate the turbidity and get a clear solution.

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Quantitative determination

Quantitative determination of nitrate: 10 mL of the transparent, clear solution was analyzed for the nitrate content using “Hi-83203 HANNA Instrument” that gives nitrate contents in samples as NO₃-N. The concentration of nitrate should be calculated by multiplying the nitrogen concentration by a factor of 4.43 in order to find the precise nitrate concentration in mg/kg of the vegetable samples [6].

Quantitative Determination of nitrite: with the AOAC official Methods 973/31. A portion of solution containing nitrite was transferred into a 25 mL volumetric flask. Then 2.5 mL sulfonamide were added, followed by addition of 2.5 mL NAD [N-(1-naphthyl) ethylenediamin 2HCl]. The volume was complete with water and left 15 minutes in order to give time for color development. The absorbance was measured at 545 nm against a blank solution. The nitrite concentration was determined using the calibration curve solutions of 0.2, 0.4, 0.6 and 0.8 ppm NaNO₂. The absorbance values were measured at 545 nm. The calibration curve was constructed by plotting the absorbance vs. the concentration.

Statistical Analysis

Values were expressed as the mean (g/kg) ± standard deviation (SD). Seasonal differences on the basis of the type of vegetables and cultivation practices (conventional vs. greenhouse) were determined by student t-test. Seasonal changes were calculated by one way Anova and for analysis of the role of multiple factors univariate analysis was used by SPSS 16. Probability values of <0.05 were considered significant.

Results

Nitrate and nitrite content in crops samples were determined in

Crops	No. of Samples	Mean(NO ₃) mg/kg ± S.E*	Range (mg/kg FW)
Iceberg Lettuce	120	2344.12 ± 21.01	1723-4788
Romania Lettuce	120	2266.65 ± 21.36	820-4586
Celery	120	3219.33 ± 79.21	3004-5011
Spinach	50	3615.19 ± 25.52	2989-5036
cabbage	120	1298.64 ± 39.49	654-2063
Chinese cabbage	80	3899.56 ± 54.12	3431-6296
cauliflower	60	241.93 ± 79.25	55-427
Potato	120	573.40 ± 25.44	237-992
carrot	120	849.28 ± 48.51	237-1045

* S.E : standard error of the mean

Table 1: Average nitrate (NO₃) content (mg/kg FW) in the crops surveyed in Tehran markets in 2011.

Crops	No. of Samples	Mean(NO ₂) mg/kg ± S.E*	Range (mg/kg FW)
Iceberg Lettuce	120	0.81 ± 0.06	0.09-1.94
Romania Lettuce	120	0.76 ± 0.03	0.07-1.83
Celery	120	3.18 ± 0.27	0.45-5.48
Spinach	50	3.58 ± 0.69	0.16-9.05
cabbage	120	1.45 ± 0.77	0.22-3.76
Chinese cabbage	80	5.55 ± 0.41	1.16-8.11
cauliflower	60	0.91 ± 0.24	0.27-1.18
Potato	120	0.88 ± 0.16	0.11-1.66
carrot	120	0.97 ± 0.11	0.05-2.45

* S.E : standard error of the mean

Table 2: Average nitrite (NO₂) content (mg/kg FW) in the crops surveyed in Tehran markets in 2011.

Tehran markets as showed in Table 1 for nitrate, and in Table 2 for nitrite.

The intakes of nitrate and nitrite from food were calculated as a global level on the basis of mean food consumption in the GEMS/Food regional diet [WHO,1998]. Intake from drinking water was added, assuming a water consumption of 2 L/day. The mean concentration in water that was used in the intake calculation was 4 mg/L for nitrate and 0.3 mg/L for nitrite which was representative of the usual concentrations found in water [WHO, 1998]. An average body weight of 60kg was used for the global intake assessment [3]. Estimated nitrate and nitrite intakes from crops according to consumption in Middle Eastern [7], due to mean concentration in our study can be seen in Tables 3 and Table 4 respectively.

Estimated nitrate and nitrite intakes from cabbage according to consumption in the Middle Eastern in our study are the highest that can be seen in Figure 1.

The nitrite levels for cabbage, iceberg and Romania Lettuces generally were below the allowable range. The nitrite content must be lower than 1 mg/kg [8,9]. The nitrite content results in this survey were lower than 1 mg/kg, except in some celery, cabbage, Chinese cabbage and Spinach samples which were higher than 1 mg/kg but still in acceptable range and propose no danger on human health as shown in Figure 2. It was reported that the nitrite levels start to be dangerous if it is higher than 100 mg/kg [2] which is not the case here.

Crops	Mean Nitrate (mg/kg)	Middle Eastern	
		Consumption (g/day)	Consumption (mg/day)
Iceberg Lettuce	2344.12	2.3	5.4
Romania Lettuce	2266.65	2.3	5.2
Celery	3219.33	0.5	1.6
Spinach	3615.19	0.5	1.8
cabbage	1298.64	5	6.5
Chinese cabbage	3899.56	0.1	0.38
cauliflower	241.93	1.3	0.31
Potato	573.40	5.9	3.4
carrot	849.28	2.8	2.4
ADI%*			20

ADI: Acceptable Daily Intake

Table 3: Nitrate intakes from crops surveyed in Tehran according to Middle Eastern consumption [7].

Crops	Mean Nitrate (mg/kg)	Middle Eastern	
		Consumption (g/day)	Consumption (mg/day)
Iceberg Lettuce	0.81	2.3	0.0019
Romania Lettuce	0.76	2.3	0.0017
Celery	3.18	0.5	0.0016
Spinach	3.58	0.5	0.0018
cabbage	1.45	5	0.0072
Chinese cabbage	5.55	0.1	0.0006
cauliflower	0.91	1.3	0.0012
Potato	0.88	5.9	0.0052
carrot	0.97	2.8	0.0027
ADI%*			50

ADI: Acceptable Daily Intake

Table 4: Nitrite intakes from crops surveyed in Tehran according to Middle Eastern consumption [7].

Conclusion

In our studies, farmers probably use higher amounts of fertilizers than they should, either due to their ignorance, or because they want to increase their production quickly when the prices of their products in markets are high. Plants cannot utilize all the added fertilizers, so the excessive amounts will be widespread by irrigation water through the soil to reach ground water, or it may dissolve in runoff water and flows into streams or takes and rivers. A highly significant, although low, positive correlation ($r=0/55$, $p=0/01$, $n=112$) was found between nitrate and nitrite contents of the winter and spring grown vegetables, compared to summer and autumn grown vegetables, although no significant variance in nitrite levels was found for most vegetables cultivated during the summer and winter.

The estimated daily intake (EDI) of nitrate from Iceberg Lettuce, Romania Lettuce, cabbage and potato was 5.4, 5.2, 6.5, 3.4 g [kg body weight (bw x d)] for adult respectively, that showed nitrate level in Lettuce and cabbage is much higher than maximum EC levels. Potential health risked from exposure to nitrate in this vegetable need more attention. These data indicates that the average intake of nitrate from most of leafy vegetables is below the acceptable daily intake, i.e. 3/7 mg nitrate ion/kg body weight per day [4], but the total intake should be monitored to protect groups at risk, such as children and vegetarians.

In accordance to the results we obtained, we highly recommend that more work to be conducted on this subject. Both governmental and nongovernmental organizations have to focus on this issue and must

consider it as part of their strategy. The education and research centers have to perform more comprehensive survey on other agricultural crops and on different production areas.

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