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Research article

INVESTIGATION OF COOKING METHOD ON NITRATE AND NITRITE CONTENTS IN CROPS AND VEGETABLES AND ASSESS THE ASSOCIATIED HEALTH RISK

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ABSTRACT: According to the recent studies southern parts of Tehran have the highest levels of pollution of environmental contaminants. A descriptive – analytical and cross-sectional study was conducted for determination nitrate and nitrite in vegetable and crops in Tehran's farmlands. 1650 samples of 13 varieties of vegetables: Potato, Onion, Carrot, Iceberg lettuce, Romania Lettuce, Celery(petioles and leaves), Cauliflower, Broccoli, Beetroot, Eggplant, Spinach, and cabbage during 12 consecutive months of 2012and 2013 cultivated in the 40 important agricultural areas in the south of Tehran were collected. The study indicated that size of crops in onion, potatoes, beetroots, carrot, cabbage and lettuces has an effect on its nitrate content as mostly in large crops the nitrate content was less. Large size onion, carrot, beetroot and potato had significantly ($p<0.01$) lower nitrate levels than small size. In accordance of the result of nitrate and nitrite content after boiling samples finding that the nitrate content were reduced with boiling by approximately: 54% in cabbage, 36% in potato, 43% in cauliflower, 19% in onion, 39% in celery and 11% in carrot samples, while the contents of nitrite in most of samples were the same and only in potato and onion were reduced with boiling approximately 9.6% and 10.3% respectively. Considering the high levels of nitrate in leafy vegetables especially lettuces, the ingestion of only 100 g of raw vegetables with the nitrate concentration of 2500 mg/kg will already lead to an intake of 250 mgNO_3. Consuming this item alone, for a person of 60 kg, would exceed the ADI for nitrate by 13%. Chinese lettuce, Iceberg Lettuce, Onion, potato, spinach samples were 100 % higher than standard levels of ECR especially they are in the highest level in the cold seasons. Nitrate levels in these vegetables and crops of southern Tehran are higher than maximum acceptable ECR levels.

Key Words: Nitrate, Nitrite, Vegetables, Crops, cooking method, Health Risk

INTRODUCTION

Increasing nitrate and nitrite contents in food is one of the most important issues that could become a global concern in polluted areas as after industrial evolution. It causes great threaten to human health, environment, plants and animals. Nitrite reacts in stomach with nitrosable compounds to form N-nitraso compounds [1,2,3] . The consumption of risk nitrate diet increased the risk of formation of carcinogenic nitrosamine [1].High levels of nitrate intake were linked with the Non-Hodgkin's lymphoma [4] , bolder cancer [4] , pancreatic cancer[5], and stomach cancer [6,7,8,9] .The US National Research Council found an association between high nitrate intake and gastric and esophageal cancer [4]. There are several factors affecting NO_3 uptake and accumulation in vegetables issues, e.g. genetic factors, environmental factors: atmospheric humidity, substrate water content, temperature, irradiance, photoperiod and agricultural factors: nitrogen doses and chemical forms, availability of other nutrients, use of herbicides, storage, etc. [10, 11, 12] . Of these factors, nitrogen fertilization and light intensity have been identified as the major factors that influence nitrate content in vegetables [13]. Limited information is available on the safety issues of crops and vegetables of Tehran where has the highest level of pollution of environmental contaminants that are related to the use of urban and industrial wastewater and agricultural practices.

According to the recent, studies southern parts of Tehran have the highest levels of pollution of environmental contaminants especially heavy metals that are related to the use of urban and industrial wastewater and agricultural practices [14,19,20,] , but to our knowledge there is no study on the nitrate levels of water ,food and soil in this area. The objectives of this study were:1- Determine the level of nitrate and nitrite contents in: Iceberg, Chinese and Romania Lettuce, Celery, Spinach, Cabbage, carrot, potato, eggplant, cabbage, cauliflower, broccoli and onion cultivated in the most important agricultural Tehran areas. 2- Determine the effect of seasons and practices on the contents of nitrate and nitrite. 3- Determine the factor of size of crops on the level of nitrate and nitrite. 4- Comparing the content of nitrate and nitrite between raw and boiling and cooking in the vegetables. 5- Assess the associated health risk posed to the Tehran population through exposure to nitrate and nitrite from these crops.

MATERIALS AND METHODS

Sampling method: A descriptive – analytical and cross-sectional study was conducted for determination nitrate and nitrite in vegetable and crops in Tehran's farmlands. 1650 samples of 13 varieties of vegetables: Potato, Onion, Carrot, Iceberg lettuce, Romania Lettuce, Celery(petioles and leaves), Cauliflower, Broccoli, Beetroot, Eggplant, Spinach, and cabbage during 12 consecutive months of 2012and 2013 cultivated in the 40 important agricultural areas in the south of Tehran were collected. 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 and nitrite content within sub-samples, five plants or bundles of sub-samples (7800 sub-samples) on the whole were analyzed separately by two ways: fresh weight and dried weight methods. In order to be sure about the accuracy of the results 148 samples were analyzed for nitrate and nitrite content by ion chromatography and colorimetric method too and following the study by boiling 120 samples of fresh and boiled celery, carrot, potatoes, onion, cabbage and cauliflower, due to find the effect of boiling and cooking on the nitrate and nitrite content.

Nitrate and nitrite Extraction: All samples (including subsamples) were rapped with plastic cover at the purchase time. All sub-samples were put into cooler boxes immediately after purchasing and washed to remove soil. Fresh weight per plant or petioles was measured. Dead leaves and non-edible parts of samples were removed and weighed. A half of each sub-sample was taken for nitrate determination and another half was used for moisture measurement. Moisture content was determined by the difference between weights before and after heating at 60 – 700 c for 48 hr.

Quantitative Determination

Quantitative determination of nitrate: For nitrate analysis, sub-samples were chapped and mixed with a food processor. Fifty to 100 grams of sub-sample were weighed and placed into a mixer. Deionized water was added to the samples (nine times than exact the sample weight) and the water and sub-sample were homogenized for 10 minutes. A 30 gram sample of homogenate was placed in a centrifuge tube, and 0.5 ml of H2O2 was added and the tube was capped and shaked well by the hand after adding H2O2. All samples were centrifuged at 3500 rpm for 3 min. The supernatant was then separated and filtered with filter paper wattman ≠ 1 and nitrate concentration in the filtrate was determined calorimetrically by a flow injection analysis system (Leif 1979). Nitrate content was expressed as mg nitrate per kg on a fresh weight basis (mg NO3/kg FW) unless otherwise stated. Nitrate concentration in celery was calculated from nitrate content in leaves and petioles separately on the weight of each part.

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 crops 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 17.Probability values of <0.05 were considered significant.

RESULTS & DISCUSSION

Mean contents and ranges of nitrate and nitrite: The different agricultural areas in the south of Tehran in this research show no significant effect on the nitrate and nitrite contents in crops and vegetables samples tested. Nitrate and nitrite mean content and their ranges in 13 vegetable crops were determined in 40 areas of the south of Tehran in 2012-2013 (4 season study) as shown in Table 1 for nitrate and in Table 2 for nitrite.

Table 1: Average nitrate (NO3) content (mg/kg FW) in the crops cultivated in the south of Tehran

Crop	**n**	**Mean (NO3) mg/kg ±S.E.***	**Range (mg/kg FW)**
Broccoli (*Brassica Oleraces*)	50	370.33±16.16	125.4-1015.32
Cabbage (*Brassica Oleraces*)	120	1272.89±39.87	575.87-2097.93
Carrot (*Daucus Carota* L.)	160	480.07±0.15	333.01-797.93
Celery (Apium graveolens)	120	3632.22±81.14	2907.69-5073.93
Chinese Lettuce (*Lactuca Sativa*)	80	3637.63±60.09	3243.32-3997.75
Cauliflower(*Brassica Oleraces*)	60	195.29±45.37	41.59-427.37
Eggplant (*Solanum melongena* L.)	84	247.16±9.69	168.97-391.08
Lettuce Romaine (*Lactuca Sativa*)	200	3585.50±27.04	1981.28-3954.69
Lettuce Iceberg(*Lactuca Sativa*)	180	3359.91±20.24	2762.46-3451.38
Onion (*Allium Cepa*)	120	999.96±17.19	712.24-1296.14
Beet root((*Beta vulgaris* L)	56	3045.54±55.50	2255.82-3646.18
Potato (*Solanum tuberosum*)	160	520.69±27.33	2255.82-3646.18
Spinach (*Pinacia oleracea*)	50	3440.73±15.66	3303.62-3646.31
*S.E.: Standard Error of the mean			

Table 2: Average nitrate (NO_2) content (mg/kg FW) in the crops cultivated in the south of Tehran

crop	**n**	**Mean (NO_2) (mg/kg FW)**	**Range (mg/kg FW)**
Broccoli (*Brassica Oleraces*)	50	0.78±0.04	0.60-1.17
Cabbage (*Brassica Oleraces*)	120	1.43±0.48	0.02-4.66
Carrot (*Daucus Carota* L.)	160	1.01±0.07	0.04-1.29
Celery (Apium graveolens)	120	3.27±0.50	0.41-6.94
Chinese Lettuce (*Lactuca Sativa*)	80	6.37±0.50	1.01-8.25
Cauliflower(*Brassica Oleraces*)	60	0.96±0.08	0.79-1.32
Eggplant (*Solanum melongena* L.)	84	0.96±0.08	0.79-1.32
Lettuce Romaine (*Lactuca Sativa*)	200	0.71±0.03	0.04-1.85
Lettuce Iceberg(*Lactuca Sativa*)	180	0.76±0.06	0.04-2.00
Onion (*Allium Cepa*)	120	4.04±0.62	1.09-9.04
Beet root((*Beta vulgaris* L)	56	1.02±0.22	0.55-1.39
Potato (*Solanum tuberosum*)	160	0.88±0.16	0.05-1.42
Spinach (*Pinacia oleracea*)	50	6.18±0.87	0.07-9.93
*S.E.: Standard Error of the mean			

Risk Assessment

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 [15]. As we have no documented data for dietary intake in Iran, nitrate and nitrite intakes from crops according to consumption in Middle Eastern [16] estimated in table 3 and 4 respectively.

Table3: Nitrate intakes from crops surveyed in Tehran according to Middle Eastern consumption.[15,16]

Crops	Mean Nitrate (mg/kg)	Middle Eastern	
		Consumption (g/day)	Consumption (mg/day)
Broccoli (*Brassica oleraces italic*)	370.33	0.5	0.185
Cabbage	1272.89	5	6.36
Carrot	480.07	2.8	1.34
Celery (Apium graveolens)	3204.54	0.5	1.60
Chinese Lettuce	3637.63	0.1	0.36
Cauliflower	195.29	1.3	0.25
Eggplant	247.16	2.8	0.69
Lettuce Romaine (*Lactuca Sativa*)	3585.50	2.3	8.25
Lettuce Iceberg	3359.91	2.3	7.73
Onion (Red)	999.96	23	23.00
Beet root	3045.54	0.5	1.52
Potato	520.69	5.9	3.07
Spinach (Spinacia oleracea)	3440.73	0.5	1.72
ADI%*			20

*ADI: Acceptable Daily Intake

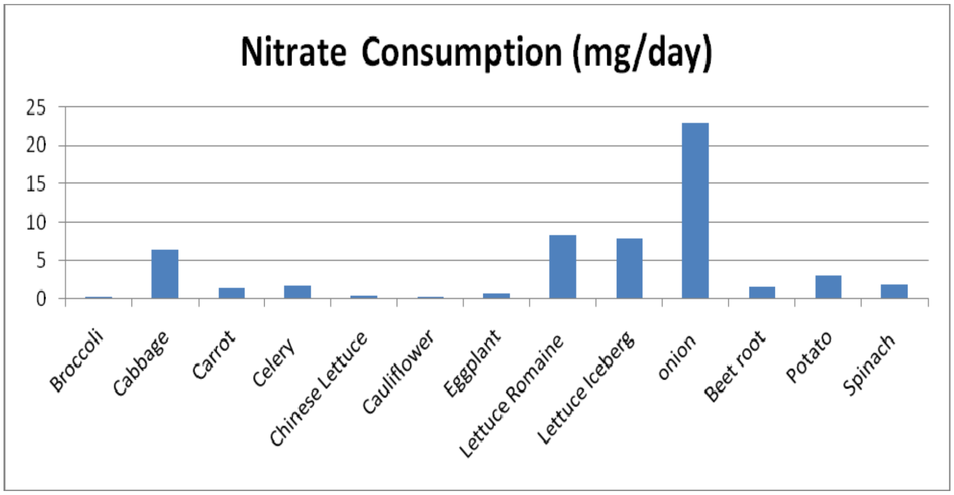
The major sources of nitrate are onion, potatoes and lettuce, due to the largest quantity mainly consumed from vegetables and crops and latter due to high nitrate content.

Table4: Nitrite intakes from crops surveyed in Tehran according to Middle Eastern consumption.[15,16]

Crops	Mean Nitrate (mg/kg)	Middle Eastern	
		Consumption (g/day)	Consumption (mg/day)
Broccoli	0.78	0.5	0.004
Cabbage	1.43	5	0.0071
Carrot	1.01	2.8	0.0028
Celery	1.53	0.5	0.0008
Chinese Lettuce	6.37	0.1	0.0006
Cauliflower	0.75	1.3	0.0010
Eggplant	0.96	2.8	0.0027
Lettuce Romaine	0.71	2.3	0.0016
Lettuce Iceberg	0.76	2.3	0.0017
Onion	4.04	23	0.0929
Beet root	1.02	0.5	0.0005
Potato	0.88	5.9	0.0052
Spinach	6.18	0.5	0.0031
ADI%*			20

*ADI: Acceptable Daily Intake

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



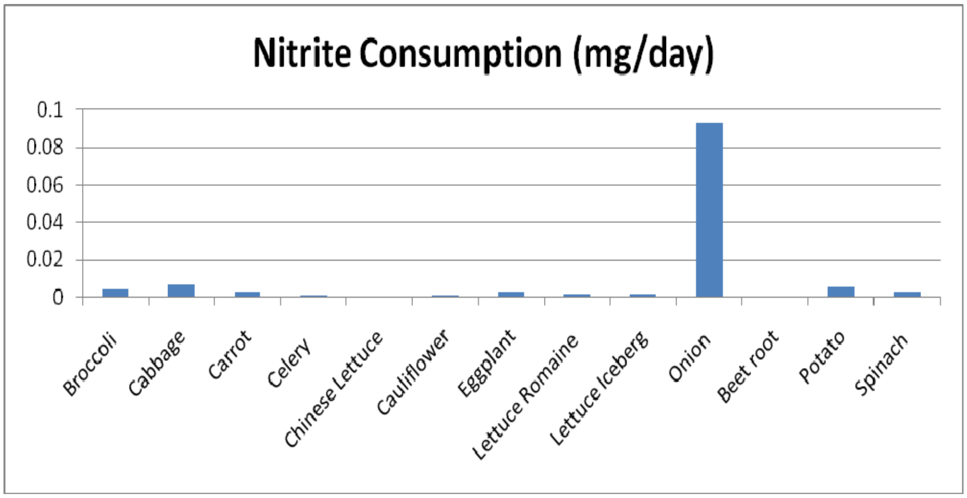


Figure-1: Estimated nitrate and nitrite intakes from crops

Results of the effect of size, practices and seasons: The study indicated that size of crops in onion, potatoes, beetroots, carrot, cabbage and lettuces has an effect on its nitrate content as mostly in large crops the nitrate content was less. Large size onion, carrot, beetroot and potato had significantly ($p<0.01$) lower nitrate levels than small size. A highly significant, although low, positive correlation ($r= 0.54$, $p=0.01$, $n=200$) was found between nitrate and nitrite contents of the greenhouse –grown leafy vegetables , compared to a non-significant and much lower correlation between the two variable in the conventional open- field- grown leafy vegetables. Winter/autumn grown samples contained significantly higher nitrate levels than other seasons ($p<0.01$).

Results of Cooking: In accordance of the result of nitrate and nitrite content after boiling 120 samples of carrot, celery, potato , onion , cabbage and cauliflower finding that the nitrate content were reduced with boiling by approximately : 54% in cabbage, 36% in potato, 43% in cauliflower, 19% in onion, 39% in celery and 11% in carrot samples, while the contents of nitrite in most of samples were the same and only in potato and onion were reduced with boiling approximately 9.6% and 10.3% respectively.

CONCLUSION & RECOMMENDATION

In particular, the ranges of nitrate levels tended to be higher in Leafy vegetable samples especially in onion in winter. In this study we found higher nitrate content in small size of most crops. Probably during initial growth, the nitrate taken up by the plant for root and shoots development process. In this stage, the roots are able to take up more nitrate than required and nitrate accumulates in the leaves and stems of the crop.

As the plants growing up, the leaves would be able to convert more nitrates into plant protein and therefore fewer nitrates can be found in the plant by maturing progress. Considering the high levels of nitrate in leafy vegetables especially lettuces, the ingestion of only 100 g of raw vegetables with the nitrate concentration of 2500 mg/kg will already lead to an intake of 250 mgNO3. Consuming this item alone, for a person of 60 kg, would exceed the ADI for nitrate by 13%. Calculating in the partial conversion of nitrate to nitrite (5%) after such consumption, the current SCF ADI for nitrite (0.06 mg/kg bw) would be exceeded by 247 %.[17,18]. Chinese lettuce, Iceberg Lettuce, Onion, potato, spinach samples were 100 % higher than standard levels of ECR especially they are in the highest level in the cold seasons. Nitrate levels in these vegetables and crops of southern Tehran are higher than maximum acceptable ECR levels , but the results of our studies of the intake of nitrate and nitrite from all dietary sources showed mean consumptions of both below the ADIs, except onion, although some consumers at high percentiles exceeded the ADI for both chemicals. Potential health risks from exposure to nitrate via onion and lettuce intake are important health concerns for Tehran's inhabitants as they are consumed in large quantities generally in most of people and needs more attention due to the largest quantity mainly consumed and latter due to high nitrate content especially for vegetarians. The quality of a plant product is determined by the prevailing conditions during growth, and accepted Good agricultural Practices (GAP) can control this [22]. In accordance of the results we obtained, we highly recommend that more study to be conducted on the nitrate and nitrite subject. Both governmental and non-governmental organizations should focus on the issue and must consider it as one of the major environmental issues. This study may propose that the over fertilization with nitrogen fertilizers in southern parts of Tehran intentionally and combustion of fossil fuels unintentionally as a by- product cause impermissible nitrate levels in leafy vegetables regardless of the kind of fertilizers [21]. It is upper than standard levels which may increase the risk of GI cancers especially stomach cancer as the most prevalent cancer in Tehran. More studies are necessary for health risk assessment regarding nitrate and nitrite intakes by food.

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