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Evaluation of the Nitrate Content in Leafy Vegetables of Southern Parts of Tehran: A Four Seasonal Study

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Abstract

Nitrate levels in water and food supplies have been increased during last decades worldwide so far the nitrate pollution even in the vegetables could become a global concern in polluted areas which may affect the human health. Limited information is available on the safety issues of leafy 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. This study aimed to evaluate the nitrate content of three leafy vegetables, assess the role of culture methods on nitrate content of leafy vegetables and compare them with the acceptable intake level of nitrate for health risk assessment. Validated standard methods of nitrate analysis were used to find nitrate concentration in 480 fresh samples from 10 different southern markets of Tehran during 12 consecutive months of 2010. Although no significant difference was recorded between the mean levels of Iceberg and Romania nitrate levels (2590mg/kg + 902.73 vs. 2302mg/kg + 959.68, p = 0.34) in general, the levels were changed significantly in every season (p = 0.01). The highest level was identified in the winter (3200 mg/kg + 1024.65) and the lowest level was identified in the summer (1946.23 mg/kg + 218.39). The levels of nitrate in the celery were not significantly changed during each season. Even in the same season and same practice, there was a wide range of nitrate content in samples. In particular, the ranges of nitrate levels tended to be higher in outdoors production than in glasshouse lettuce samples. Nitrate levels in lettuces of southern Tehran are higher than maximum acceptable ECR levels. Potential health risks from exposure to nitrate via lettuce intake are important health concerns for Tehran's inhabitants and needs more attention.health problem among Pakistani children due to changes in life style and other factors. This study is aimed to estimate the prevalence of obesity and the associated factors leading to obesity among school students in the urban city of Hyderabad.

Keywords: Nitrate; Leafy vegetables; Lettuce; Celery; Tehran

Introduction

Nitrate levels in water and food supplies have been increased during last decades worldwide so far the nitrate pollution has become a global concern which may affect the food quality for daily use and impair the human health [1]. Analysis of pollution sources of nitrate shows that the heavily polluted regions are usually associated with larger uses of nitrogen fertilizer and household livestock or poultry [2].This situation is existing for southern parts of Tehran where provides the major part of the leafy vegetables of Tehran province. According to the recent, studies southern parts of Tehran has 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 [3] but to our knowledge there is no study on the nitrate levels of water ,food and soil in this area.

The potential hazard of vegetable-borne nitrate is from its conversion to methaemoglobin-producing nitrite before and/or after ingestion [4]. Nitrates and nitrites, under normal gastric conditions, are known to be precursors to Nitroso compound formation in the presence of secondary or tertiary amines or amides [5]. Approximately 5% of ingested nitrates in food and water are converted to nitrite in the saliva, further promoting endogenous nitrosamine formation [6]. Approximately 80% of dietary nitrates are derived from vegetable consumption [7,8]. The sources of nitrites include vegetables, fruit, and processed meats therefore additional studies on the level of nitrate in food and water in populations with well-characterized exposures are urgently needed to further our understanding of cancer risk associated with nitrate ingestion [9]. We aimed in the present study to detect the nitrate levels in the heads and leaves of Iceberg Lettuce, Romania Lettuce and Celery because these vegetables are widely consumed across the country and people use them to prepare different salads, some ethnic cuisine, and garnishes and Iranian habitants use them routinely freshly in uncooked foods in large amounts in all four seasons.

Materials and Methods

Subjects and sampling method:

Three types of vegetables were purchased at the farmers market in the south of Tehran. The farmers market also offered the chance to speak with growers directly to obtain information regarding their farming practices. As farmers cultured these vegetables by two different methods in all seasons (Conventional/outdoors production and greenhouse/glass house), both farming practices were considered in this study. For each season the sampling and analysis were repeated six times in two equal intervals (15 days) each contained 5 subsamples and the nitrate levels of each subsamples were determined two times by 2 ways, fresh weight and dried weight methods. Table 1 shows the sampling method of this study. To evaluate the variability of nitrate content within sub-samples, five plants or bundles of sub-samples were

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Factors	Sub-factors
Type of vegetable (3)	Iceberg lettuce, Romania lettuce ,Celery*
Season (2 x2x2x2x2)	Winter ,Spring ,Summer ,autumn{ 2times repeated per season}**
Origins(3)	3 different farms (per practice)
Sample (5)	5 sub-samples (plants)

*Head and leafs were analyzed separately.
** Leaf blades and petioles were analyzed separately.

Table 1: Sampling method of market survey.

analyzed separately and averaged to produce one sample data point which has been summarized in table 1.

Pretreatment and nitrate analysis

Both outdoors and glasshouse vegetables were purchased on the same day and 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 for lettuce or petioles for celery was measured. Dead leaves and non-edible parts of samples were removed and weighed. A half lettuce or celery 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 – 70°c for 48 hr. 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 H_2O_2 was added and the tube was capped and shaked well by the hand after adding H_2O_2. 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 [10]. Nitrate content was expressed as mg nitrate per kg on a fresh weight basis (mg NO_3/kg FW) unless otherwise stated. Nitrate concentration in celery as a whole plant was calculated from nitrate content in leaves and petiols and the weight of each part.

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. Coefficients of variation (CV = standard deviation / average × 100) were calculated to indicate variation within sub-samples and factors. Values for average moisture content were calculated by % w/w.

Iceberg	Season	Practice	n	NO_3 mg/Kg Fw				NO_3%DW			
				Ave.	Min.	Max.	CV%	Ave.	Min.	Max.	CV%
Iceberg	winter	Outdoors	6	3654	2706	4788	35	2.25	1.67	2.95	16
		Glasshouse	6	2234	1987	3831	32	2.15	1.59	2.71	25
	Winter total		12	2944	1987	4788	34	2.20	1.59	2.95	19
	Spring	Outdoors	6	2230	2130	3400	30	2.01	1.86	2.54	9.7
		Glasshouse	6	1977	1723	2406	18	2.33	1.76	2.34	11
	Spring total		12	2104	1723	3400	25	2.17	1.76	2.54	10
	Summer	Outdoors	6	1970	1870	2100	8	1.98	1.27	1.96	30
		Glasshouse	6	1677	1760	2300	23	1.88	1.32	1.81	31
	Summer total		12	1824	1760	2300	15	1.93	1.27	1.96	29
	Autumn	Outdoors	6	3010	2238	4507	30	2.19	1.57	2.88	25
		Glasshouse	6	2005	1878	3778	28	2.11	1.51	2.45	62
	Autumn total		12	2508	1878	4507	29	2.15	1.51	2.88	43
	Outdoors produced total		24	2716	1870	4788	26	2.05	1.27	2.71	23
	Glasshouse produced total		24	1973	1723	3831	25.2	2.15	1.32	2.95	28
Romania	Iceberg total		48	2344	1723	4788	25.5	2.10	1.27	2.95	26
	Season	Practice	n	mgNO_3/KgFw				NO_3%DW			
				Ave.	Min.	Max.	CV%	Ave.	Min.	Max.	CV%
	Winter total		12	3073	2276	4586	32	2.54	0.92	3.68	37
	Spring	Outdoors	6	2142	2011	3400	30	2.21	2.09	3.22	19
		Glasshouse	6	1980	1780	2800	21	2.16	0.65	3.01	41
	Spring total		12	2061	1780	3400	26	2.18	0.65	3.22	30
	Summer	Outdoors	6	1030	890	1200	13	1.94	1.70	3.24	11
		Glasshouse	6	1170	820	1500	23	1.99	1.32	2.85	29
	Summer total		12	1100	820	1500	20	1.96	1.32	2.85	21
	Autumn	Outdoors	6	2987	2010	3122	11	2.33	2.01	3.01	21
		Glass house	6	2055	1776	3088	29	2.01	1.22	2.94	43
	Autumn total		12	2521	1776	3122	21	2.17	1.22	3.01	33
	Outdoors total		24	2420	890	3986	22	2.23	1.70	3.68	18
	Glasshouse total		24	1957	820	3186	26	2.10	0.92	3.47	42
	Romaine total		48	2188	820	4586	24	2.16	0.92	3.68	30
	Grand total		96	2266	820	4788	25	2.13	0.92	3.68	28

Table 2: Nitrate content in lettuce samples: Four seasonal determinations.

Results

The total levels of nitrate were determined in two types of lettuces and celery in four seasons of 2010 in Tehran. The mean level in Iceberg was 2590 + 902.73 mg/kg and the mean level in Romania was 2302 + 959.68 without any significant difference (p=0.34). During the second half of the 20h century, production of human made nitrogen compounds were increased dramatically intentionally through the use of fertilizers or unintentionally as a by- product of the combustion of fossil fuels [11]. Both sources of pollution are existing in the southern parts of Tehran were these samples were cultured. General features of nitrate content of vegetables have been summarized in table 2.

Many data exist on the concentration of nitrate in vegetables but most of them are more than 20 years old and produced from few countries. Reported levels were ranged from 30 to 6000 mg/kg [11-14]. We have reported the levels of nitrate in leafy vegetables for the first time in Tehran which is the capital city of Iran and one the most polluted cities worldwide [15]. In total, during the current Iranian calendar year which starts on March 21 every year, air pollutants in Tehran have exceeded the standard levels for over 33 days [16, 17].

Seasonal differences in nitrate levels of both types of lettuce (Iceberg and Romania)

The levels of nitrates were differentially determined by the type of vegetable, season and cultivation style by two methods of analysis that means fresh weight and dried weight. The levels were changed significantly in every season (p= 0.01). The highest level was identified in the winter (3200 mcg/g + 1024.65) and the lowest level was identified in the summer (1946.23 + 218.39). Table 3 compares the total levels among all seasons. According to our study nitrate levels in Lettuce ranged from 1946.23(218.39) mg/kg in the summer to 3200.03(1024.65) in the winter (Table 3) which is the most polluted season every year especially in the winter 2010 [15, 16].

The estimated annual amount of air pollutants in Iran has been reported as 5 million tons [18]. In Tehran, the capital of Iran, air pollution occasionally reaches dangerous levels particularly during the cold season because of the phenomenon known as temperature inversion. Over the past three decades, air pollution in Tehran has been regarded as a multifaceted problem [15]. The combustion of substandard fossil fuels such as leaded gasoline also enhances air pollution. Temperature inversion is another contributing factor, particularly during cold seasons. The production of human made nitrogen compounds were increased dramatically intentionally through the use of fertilizers or unintentionally as a by- product of the combustion of fossil fuels and both of these activities increase in cold seasons in Tehran which have affected directly the nitrate levels in two types of lettuces according to this study.

Seasons	N	Mean	Std. Deviation	Std. Error Mean	P-value
Nitrate levels(mg/kg) Spring	60	2311.0	554.57	160.09	
Summer	60	1946.23	218.39	63.04	0.045*
Autumn	60	2902.73	1013.46	292.56	0.090
Winter	60	3200.03	1024.65	295.79	0.015*
Summer	60	1946.23	218.39	63.04	
Spring	60	2311.0	554.57	160.09	0.045*
Autumn	60	2902.73	1013.46	292.56	0.004**
Winter	60	3200.03	1024.65	295.79	0.0001***
Autumn	60	2902.73	1013.46	292.56295	0.482
Winter	60	3200.03	1024.65	295.79328	

Table 3: Comparison of the total nitrate levels (mg/kg) among four seasons.

In the next study we compared the nitrate levels between Iceberg and Romania in four seasons according to the cultivation method. As we showed in Figure 1, the highest levels were indentified in Iceberg in autumn and Romania in winter by outdoor method of culture. Winter/autumn grown samples contained significantly higher nitrate levels than other seasons (p<0.01), and moisture content in winter lettuce was greater than that found in other seasons too. Environmental factors such as light intensity, air temperature, soil temperature, fertility and moisture also affect nitrate levels in vegetables. We can assume also that temperature is one of the other factors affecting nitrate levels in lettuce.

It should be noted that even in the same season and same practice, there was a wide range of nitrate content in samples. In particular, the ranges of nitrate levels tended to be higher in outdoors production (conventional) than in glasshouse lettuce (green house) samples (Figure 1).

Nitrate content of celery

The averages of nitrate levels of leaves and petiols of celery samples in different seasons are shown in Figure 2. The levels of nitrate in the celery were not significantly changed during each season. In this cross sectional study, no significant difference in nitrate levels of celery

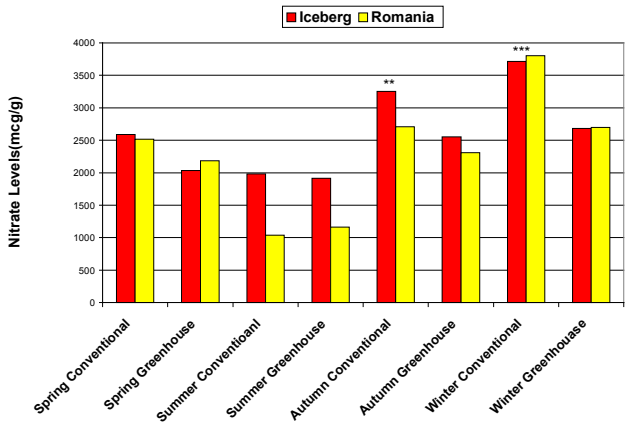

Figure 1: Comparison of nitrate levels in Iceberg and Romania lettuce samples according to the season and cultivation methods.

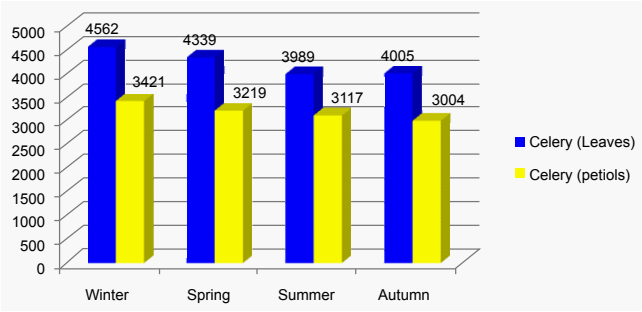

Figure 2: Nitrate levels in Celery (NO_3 mcg/g FW) in four seasons.

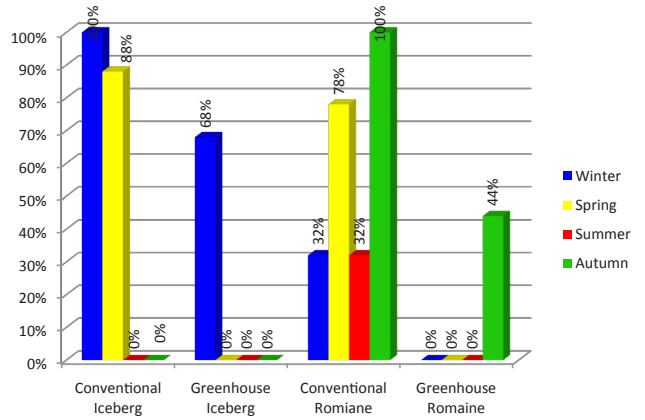

Figure 3: Nitrate levels of lettuce exceed maximum permissible levels from European Commission Regulation.

samples was determined. Although nitrate concentration in celery samples from this study ranged from 3004 to 3421 in petioles and 3989 to 4562 mg NO_3/kg FW in leaves, these levels were below the permissible limit of 5000 mg/kg for leaves and 4000 for petioles which were notified by ECR [14]. It seems that air pollution and nitrogen fertilizers could not affect the nitrate levels in celery samples significantly .Celery is offered by the literature as a safe vegetable with anticancer effects [19] even when is cultured in polluted areas like southern parts of Tehran.

Comparing the levels with maximum permissible levels according to the European Commission Regulation(ECR)

Nitrate contents in lettuces from various origins and seasons in the southern parts of Tehran in 2010 was higher than the maximum levels defined by European Commission Regulation (EC) No1881/2006 [14] as we described in Figure 3. Unfortunately 34% of all lettuce samples contained nitrate levels exceeding the maximum EC standards of ECR. Winter-grown Iceberg lettuce in particular tended to exceed the maximum levels, with 100% of outdoors and 68% of glasshouse samples. In fact the incidence rates of stomach cancer in Iran is very high, well above the world average and the incidence of gastric cancer has increased about two fold during the last decade [8].

Conclusion

In particular, the ranges of nitrate levels tended to be higher in outdoors production than in glasshouse lettuce samples especially in the winter. Although lettuce and celery are categorized as high nitrate (> 1000 mg/kg) leafy vegetables [11], the concentrations of nitrate in lettuce samples were 100% higher than standard levels of ECR especially in the cold seasons. Nitrate levels in lettuces of southern Tehran are higher than maximum acceptable ECR levels. Potential health risks from exposure to nitrate via lettuce intake are important health concerns for Tehran's inhabitants and needs more attention. In lettuces produced outdoors in the winter the levels exceeds 3500 mg/kg which was significantly higher than other seasons and cultivation method.

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. 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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