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Health Risk Assessment and Evaluation of Nitrate and Nitrite in Salad Vegetables of Mashhad City

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ABSTRACT

Introduction: Nitrate and nitrite are parts of the nitrogen cycle and can enter human body through eating and drinking. In spite of the fact that nitrate is characterized as a safe compound, it may turn into nitrite form in the body and react with amines to form dangerous compounds like nitrosamines, preventive cautions; therefore, the intake of nitrate should be controlled.

The aim of this research was to analyze data and risk assessment of nitrate/nitrite content in four commonly used salad vegetables (onion, cucumber, lettuce, and, tomato) that were sold in the Mashhad central vegetable market.

Methods: In this study, data from 1008 samples between 21st March 2019 to 20th March 2022 that were tested based on spectrophotometric method were analyzed by SPSS version 22 and compared with the Iranian National Standard and WHO limits.

Results: Nitrate and Nitrite mean values of four analyzed vegetables were: onion = 76±6 mg/ kg; 1.45±0.99 mg/kg, tomato = 95±15 Mg/kg; 2.35±1.28 Mg/kg cucumber 216±30 Mg/kg, 2.50 ±1.30 mg/kg, lettuce 1050±234 mg/kg;2.89±1.54 respectively. There was an increasing trend in nitrate levels over the 3 surveyed years. The Mean increase was significantly different between vegetables in 3 years ($p<0.01$). Mean daily intake of nitrates from vegetables was within acceptable daily intake (ADI) limit and the Noncarcinogenic index was less than EPA limits for children and adults. Target hazard quotient (THQ) and estimated daily intake (EDI) of nitrite and nitrate were less than the EDI value (1 mg/kg bw/day) and 3.70 mg/kg bw/day, respectively. But lettuce consumption should be controlled among children (THQ=0.95).

Conclusion: Nitric compounds intake from salad vegetables is considered safe for Iranian consumers. However, the EDI of nitrate from other sources, including processed meat products and drinking water, should be regarded.

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Introduction

In recent years the "Covid 19" breakout, induced the increasing consumption of vegetables as a good source of antioxidants(1). Meanwhile, vegetables are the major origins of nitrate and nitrite and nitrate entrance through comestibles can lead to human body damage(2, 3). Also, today's one great ecological issue is nitrate pollution. Accumulation of nitrate and nitrite in the body threatens human health, and problems such as cancers, digestion and absorption disorders, and even death in children may occur (4). Nitrogen cycle is the source of nitrate and its derivatives and they can infare in water and food via preservatives added to food, fossil fuels, and fertilizers (5)

In spite of the fact that nitrate is characterized as a safe compound, since it is converted to nitrite form in the body, which may react with amines to form dangerous compounds like nitrosamines, preventive cautions, the intake of nitrates should be controlled(6, 7). These compounds can cause methemoglobinemia in newborns and gastrointestinal cancer in adults and further make mutagenic and teratogenic deteriorations (8). Confrontation to nitrate derivatives also may occur by consumption of drinking water, processed meat, or vegetables (9), on the other hand, nitrate can enter drinking water through human resources. The most important sources of nitrate in the environment and drinking water are agricultural and non-agricultural

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sources(10). But around 80% of nitrates uptake is from fruits and vegetables(11).
To calculate the mean intake of nitrite and nitrate, the use of each current salad vegetable per day (kg/day) is multiplied by the median value of nitrate (mg/kg fresh weight) in that type of vegetable, and the results are expressed in mg/kg body weight/day(12).
Target hazard quotient (THQ) is computed as the ratio of confronting to the injurious stuff and the reference dose which is the largest superficies at which
no antagonistic wellbeing impacts are anticipate d. THQ > 1 means that Noncarcinogenic risk is not acceptable (13).
This research was established to evaluate nitrate and nitrite contents in 4 commonly selected vegetables and analyzed their health risk effects on the population of Mashhad city.

Material and Methods

Sampling

In this study 1008 fresh salad vegetable samples, including onions, tomatoes, cucumbers, and lettuce, were randomly collected between March 2019 and March 2022 from the main fruit and vegetable market located in Mashhad province of Iran based on the following sampling plan; Monthly, each month 7 samples were randomly selected from the sellers (2 kg of each sample) and totally 1008 vegetable samples were collected during 3 years.
After recording each sample's information in one predesigned form, including the sampling date and harvest time, samples were placed in clean and inert containers (i.e. plastic bags and containers to protect them from cross-contamination, damage, and leakage). They were analyzed immediately and if it was not possible to test quickly, they were packed in plastic wrap and stored in the refrigerator.

Chemicals

Nitrate Measurement

Extraction of the sample with hot water was performed following the precipitation of proteins by solutions of potassium hexacyanoferrate and zinc acetate. The obtained solution was filtered and the nitrate was reduced to nitrite by metal cadmium, sulfanilamide chloride, and N - 1 Ethylene diamide dihydrochloride then filtered and the red (purple-pink) complex formed by nitrite was measured at 538 nm by spectrophotometer(14, 15).

Nitrite Measurement

Potassium hexacyanoferrate and zinc acetate were used for the extraction and precipitation of proteins of the sample with hot water. After filtration of the obtained solution, sulfanilamide chloride and N- (1-naphthyl) ethylenediamine dihydrochloride was added to the filtered solution and a red complex was generated by nitrite at a wavelength of 538 nm by spectrophotometer was measured(14, 15).

Risk Assessment Method

The health risks related to the consumption of vegetables were displayed in Tables 3 and 4.
To assess the risk, the concentration of these contaminants in each type of vegetable and the bulk of vegetable intake by each person per day were calculated as a base. Then, the risk potential and risk index for health risk assessment were determined. Risk of noncancerous effects was also assessed by gaining the non-cancer risk factor (NHQ). The health risk of exposure to nitrate and nitrite for an adult person[1] and children[2] due to the consumption of vegetables was determined by the following Equation1 (3).

$$EDI = \frac{EF * ED * DC * MC}{bw * AT} \qquad \textbf{(Equation: 1)}$$

EDI stands for "estimated daily intake" (mg/kg bw/day); EF: "exposure frequency" (365 days/year); ED: exposure duration for an adult (70 years for adults and 6 years for children); DC: Daily Consumption. Based on references, per capita consumption of cucumber, tomato, onion, and lettuce, was 109, 109, 39, and 58, g/day for Iranian adult consumers and half of these for children (3, 12), MC: Mean Concentration of nitrite and nitrate (mg/kg wet weight); bw: Mean body Weight of adult consumers aged 16–70 years (70 kg); and children aged 6 years (20 kg). AT: Average Time 25550 days for adults and 2190 days for children (16, 17).

Hazard Quotient

Noncarcinogenic risk was calculated via the Target Hazard Quotient (THQ) used by previous researchers (18).

[1] 70years old and 70 kg body weight

[2] 6 years old and 20 kg body weight

Total Hazard Quotient (THQ) was obtained by Equation 2.

$$THQ = \frac{EDI}{Rfd}$$ **(Equation: 2)**

In this formula, Rfd 1.6 is the reference dose of nitrate (mg/kg bw/day), and 0.1 is used for nitrite according to the risk information system proposed by the United States Environmental Protection Agency(19).

If THQ was calculated less than 1, it shows that it is in the acceptable range of risk level for chronic health risk, and if THQ was higher than 1, it indicates that the non-carcinogenesis risk is not at a suitable level(20), there is no carcinogenic effect defined for nitrate and nitrite defined yet(21, 22).

Total Non-carcinogenic risk is calculated based on the following equation:

Total THQ (HI) = ∑THQ (Equation: 3)

Statistical Analysis

The outputs were analyzed by the SPSS software (version 22.0). At first, to examine the normality of data, a Kolmogorov–Smirnov test was used. Then a k- independent sample test was used to examine the significant relationship between nitrite/nitrate levels of vegetables. Plots were illustrated by Excel 2016. Comparisons between years and seasons were done by statistical Kruskal – Wallis test and $P<0.05$ was considered as statistical significant difference.

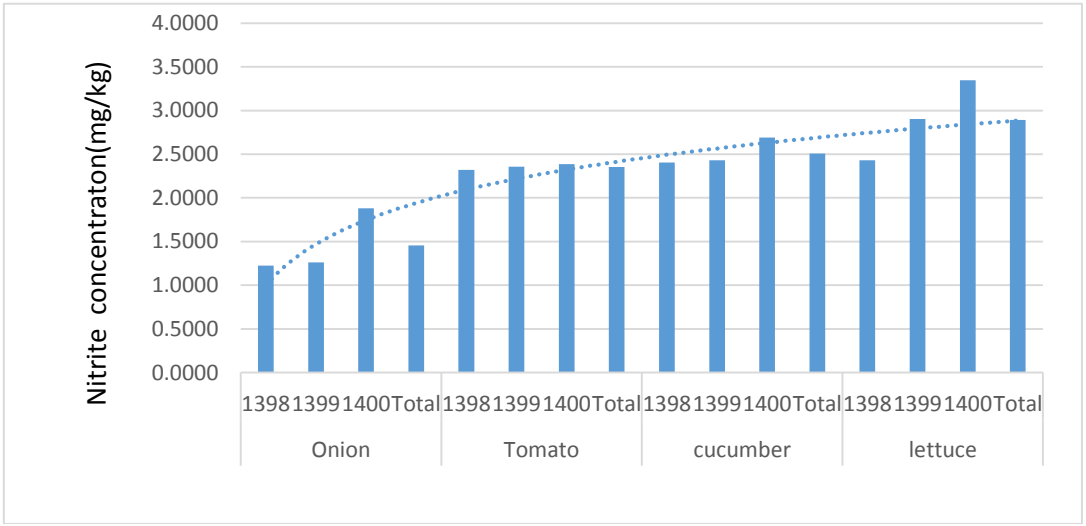

Figure 1. Nitrite concentration (mg/kg) comparison in 4 salad vegetables in Mashhad, Iran

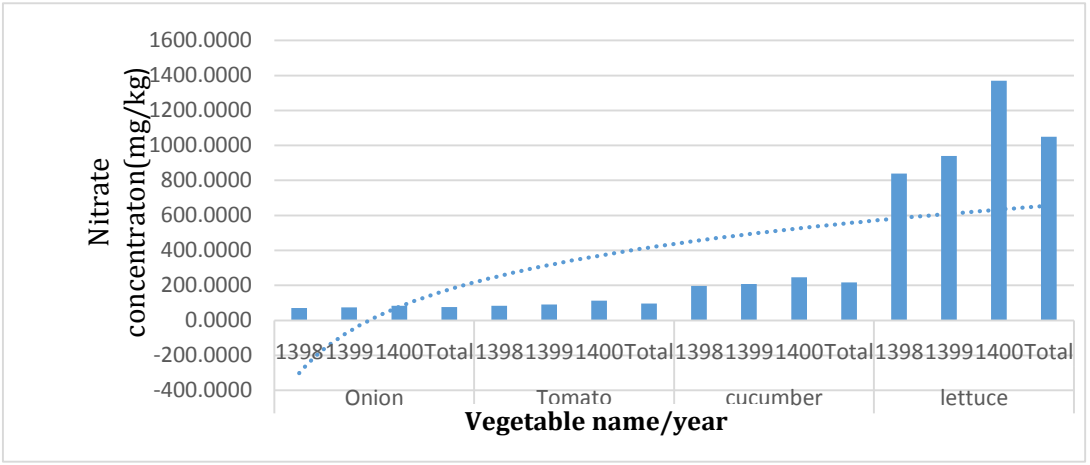

Figure 2. Nitrate concentration (mg/kg) comparison in 4 salad vegetables in 3 years in Mashhad, Iran

Results

Nitrite

As can be seen in figure 1, a relatively high difference in the concentration of nitrite was recorded between and within the different types of vegetables. The mean concentration of nitrite in different vegetables in 3 years data, can be summarized as followed; onion (1.45 ± 0.99mgkg_1), tomato (2.35 ± 1.28mgkg_1, cucumber (2.50 ± 1.30mgkg_1), and lettuce (2.89 ±1.54mgkg_1), respectively.

Nitrate

As can be seen in figure 2, relatively high variability was recorded considering the concentration of nitrite between different types of crops (p value<0.05). In this regard, the mean concentrations of nitrate in different groups of vegetables, can be summarized as onion (76.15 ± 6.83mgkg_1), tomato (95.90 ± 15.65mgkg_1), cucumber (216.93 ± 30.24mgkg_1), and lettuce (1050.06 ±234.56mgkg_1), respectively.

As is shown in figure 2 the nitrate mean in lettuce was the highest compared to other vegetables.

Risk Assessment

Results of risk assessments are shown in tables 1 and 2.

Table 1. Nitrite Estimated daily intake and THQ of nitrates through 4 vegetables consumed in Mashhad (3 years average concentration)

Vegetable	Mean nitrite content (mg/kg)	Per capita consumption (g/day)	Mean nitrite intake (mg/ day/person)	EDI Child(mg/kg bw/day d)	EDI Adult(mg/kg bw/day)	RfD (mg/kg bw/day)	THQ CHILD	THQ ADULT
Onion	1.45	39	0.92	0.001	0.005	0.1	0.01	0.05
Tomato	2.35	109	2.56	0.005	0.003	0.1	0.05	0.03
Cucumber	2.50	109	2.72	0.007	0.006	0.1	0.07	0.06
Lettuce	2.89	58	1.67	0.004	0.002	0.1	0.04	0.02

Table 2. Nitrate Estimated daily intake and THQ of nitrates through 4 vegetables consumed in Mashhad (3 years average concentration)

Vegetable /standard limit	Mean nitrate content (mg/kg	Per capita consumption (g/day)	Mean nitrate intake (mg/ day/person)	EDI Child(mg/kg bw/day	EDI Adult(mg/kg bw/day)	RfD (mg/kg bw/day)	THQ CHILD	THQ ADULT
Onion/90	76.15	39	2.97	0.07	0.04	1.6	0.04	0.02
Tomato/120	95.90	109	10.45	0.26	0.15	1.6	0.02	0.09
Cucumber/300	217.06	109	23.65	0.06	0.03	1.6	0.04	0.02
Lettuce/1500	1050.06	58	60.94	1.52	0.87	1.6	0.95	0.54

Discussion

This study intended to evaluate the nitrate/nitrate concentration level mean in the 4 most commonly used salad vegetables sold in Mashhad central fruit and vegetable. Accordingly, nitrite and nitrate levels of 1008 samples of vegetables including onion, lettuce, tomato, and cucumber, was determined.

Nitrite and Nitrate Concentration

2.50 ± 1.30 mg/kg, lettuce 2.89 ± 1.54 mg/kg; respectively. Currently, there is no defined limit for nitrite content in these 4 salad vegetables in existent national or international standards. The mean nitrite levels in these crops ranged; onion 1.45 ± 0.99 mg/kg, tomato, 2.35 ± 1.28mg/kg cucumber

Nitrite level in onion (1.45mg/kg) was more than 0.49 mg/kg.in Bahadoran et.al results (3) and the mean nitrite content in cucumber samples was 2.5mg/kg which is higher than 2.1 mg/kg in Roshana et al 2021 study (20) and more than 0.57 mg/k in Bahadoran et al (3) study.

Our result indicated that nitrite level in lettuce (2.89mg/kg) was more than 0.54mg/kg reported in the study by Bahadoran et.al (3) and was approximately similar to the reported 2.6 mg/kg in studies by Correia et al (2010) and Roshana et al (2021) (20, 23).

Our results indicated that nitrite concentration in all vegetables was very low and was directly related to their nitrate content. Mean Nitrite levels in lettuce was greater than other vegetables. Comparison of the samples obtained from three years of evaluation showed that the nitrate level increased during the three years. Our findings also showed no significant difference in pollutants between different months and seasons of the year, and the difference in the amount of pollutants in three years was not significant because of a wide range

of crop cultivation (45 different geographical regions).

Nitrate content of onion in this study (76mg/kg) was lower than the standards limits (90 mg/kg), but comparison between 3 years showed a significant increase in nitrate content (p value<0.05). Anyway, it might be due to the increased use of nitrate fertilizers during the 3 years.

Nitrate mean concentration in other samples including onion (82.25 mg kg-1) and tomato (110.93 mg kg-1) was greater compared to similar studies, such as the study by Mehri et. al(24).

In our study Lettuce with mean nitrate concentration of 1050.06 mg/ kg had the highest level of nitrate among other vegetables. But these results were lower than the studies performed in Taiwan (1520 mg/ kg) (25); in UK (2330 mg/kg) (26); and in Iran (3637 mg/kg)(27).

Mean nitrate concentration was not significantly different between seasons and months in all 4 vegetables (p>0.05).

Nitrate content in vegetables can be affected by various factors like vegetation time, temperature, soil type, the density of vegetables in the field, crop maturity, harvest interval, humidity, and fertilizers (7).

Risk Assessment

The risk for nitrate and nitrite ingestion via vegetables depends on the type of vegetables, environmental condition, farming methods, soil nature as well as the status and period of product storage and/or food processing (28).

The nitrite THQ WAS very lower than index and there is no worry about this in these vegetables.

The largest nitrate THQ was related to lettuce and the lowest amount was shown in tomatoes in children, but in adults it was lettuce> tomato> cucumber= onion.

THQ of lettuce for children (0.95) was near the standard limit (1). Therefore, it is recommended to control lettuce consumption in children less than 6 years old.

Hence, local people are not at carcinogenic risk due to the ingestion of these four vegetable crops.

The hazard index caused by both contaminants was less than 1, and no adverse effect in terms of health risk was considered.

Total exposures resulting from these 4 crops and hazard index values of children aged 6 years were found significantly higher than adults (p-value < 0. 05).

Nitrite EDI values for children decreased in the subsequent order: lettuce> > tomato > cucumber >onion. The lettuce sample had the highest EDI value (1.52 mg/kg bw/day) and while the onion sample had the lowest EDI value (0.07 mg/kg bw/day). Onion EDI was lower, and lettuce was higher in comparison with Mehri et al(24).

Some factors are associated with the health risk of nitrate and nitrite, like consumption rate, the intrinsic risk caused by nitrates and nitrite, and body weight. Our results implied that THQ values for each of the samples in this study were less than 1.

Conclusion

Our results showed that the mean nitrate concentration was less than the permissible limit in all vegetable samples.

Comparison of nitrate levels between 3 years showed an increasing trend in 4 vegetables (p<0.01) that might be due to the increasing use of fertilizers.

However, no significant difference was seen between seasons and months, vegetable type, and pollutants (p>0.05).

Health risks associated with both nitrate and nitrite were less than 1, and no adverse effect in terms of health risk was considered.

Therefore, the intake of nitrate through such food could be considered safe for Iranian consumers.

However, it would be noted that other sources such as water and processed meat products must be regarded.

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Conflict of Interest

None

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