A survey on nitrate level in vegetables to assess the potential health risks in Iran

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ABSTRACT
The present study aims to assess the nitrate level of vegetables as the major sources of nitrates in the human diet as well as the health risks resulting from their consumption in Iran. Therefore, 332 samples were randomly selected to determine the nitrate level in accordance with European Regulation (EC) No 194/97 through ion chromatography with suppressed conductivity detector. The data showed the extremely variable level of nitrate ranging from 2 to 1950 mg.kg⁻¹ in different vegetables. The highest nitrate median level was observed in spinach (393 mg.kg⁻¹) and lettuce (283 mg.kg⁻¹) and the lowest level, which was less than the permissible limit approved by the Iranian national standard organization (INSO) was found in shallot and carrot (Except to one cucumber sample). The health risk index (HRI) of nitrate, which was calculated based on the estimated daily intake (EDI) in all tested samples, was in the acceptable range, indicating that the nitrate intake resulting from vegetable consumption does not have any health risk for the Iranian population. However, it the necessity to be monitored regular surveillance of nitrate level in vegetables.

INTRODUCTION

Nitrates are water-soluble inorganic chemical compounds naturally found in various foods such as fruits and vegetables.[1–3] Unfortunately, as dietary inorganic nitrates are known to have adverse effects, strict regulations have been applied on the allowable level of nitrate in the food consumed.[4] The available evidence has proposed the positive correlation between high nitrate consumption and increased risk of Stomach and esophageal cancers resulting from the nitrosamines formation.[5,6] Contrary to popular misconception, several reports have been presented on the preventive impacts and health benefits of nitrate through lowering of blood pressure.[7]

Vegetables have been widely consumed because they contain ascorbic acid compounds, carotenoids, vitamins E and C, polyphenol, minerals, and fiber, which facilitate reduction of toxic nitrite to beneficial nitric oxide and decrease nitrosamines formation.[8,9] Based on dual effects of nitrate, the concepts of risk and benefit of exposure to dietary nitrate have become a challenging/unresolved issue to the scientists.

Vegetables are an important source of dietary exposure to nitrate which lowers the risk of cardiovascular disease, cancer, and mortality.[4] Thus, it is recommended that at least 400 g of fruits...
and vegetables be used every day in accordance with the WHO (World Health Organization) and FAO (Food and Agriculture Organization) agency.\textsuperscript{[2]} Although there is no reports about the consumption rate of vegetables in Iran (lower/higher than the WHO norms), the available evidence (non-official statics) implied low consumption of vegetables in Iran. Therefore, it is required to encourage of wider Iranians population to consume of more vegetables.

In spite of the important role of micronutrient, nitrate pollution resulting from chemical fertilizers and pesticide residue overload in vegetables and fruits has turned into a global concern.\textsuperscript{[10,11]} Based on literature, people intake almost 80\% of nitrate from vegetables.\textsuperscript{[12,13]} The possible adverse effect of dietary nitrate and nitrite intakes on health attracted the attention of many researchers.\textsuperscript{[14]} Large amounts of nitrate may be accumulated in most vegetables depending on the biological features of the plant, type of soil, planting/seeding density, (day) light intensity, temperature, humidity, vegetation period, plant maturity, source of nitrogen, size of the vegetable unit, harvesting time, and storage time.\textsuperscript{[15]}

Studies have shown that exposure to nitrites usually occurs due to the consumption of processed meat products and the rate of conversion of nitrates to nitrites in vegetables is very limited. In other words, according to the European Normative guidelines, the presence of nitrite residues in vegetables is not acceptable at any level.\textsuperscript{[16]} With increasing population in today’s societies, the need for food has increased, so the use of chemical and organic fertilizers has increased to improve production efficiency. When the amount of reduction of nitrates decreases compared to their absorption in plants, the amount of its accumulation by plants increases.\textsuperscript{[17]} Excessive use of nitrogenous fertilizers leads to the absorption and accumulation of soil nitrogen as nitrate in plants. Apart from rare side effects in some susceptible people, nitrates in themselves are not harmful to humans.\textsuperscript{[18]} In reaction with nitrifiers in the stomach, consumed nitrate changes into nitrite. The nitrate ion has a low level of acute toxicity. However, when it is converted into nitrite, it can cause health hazards in the presence of bacteria or enzyme nitrate reductase and in contact with metals.\textsuperscript{[19]}

Reduction to nitrite may results in the formation of mutagenic and carcinogenic Nitrosamines due to the reaction of nitrite with different proteins in the digestive system.\textsuperscript{[15,20]} Moreover, 5\% of the nitrate from the diet was estimated to be reduced to nitrite by the microflora in the oral cavity.\textsuperscript{[21]} As epidemiology and clinical studies show, high level of nitrate and nitrite dietary intakes have been associated with the etiology of human gastric cancer.\textsuperscript{[22]}

The other main subject related to nitrate assessment is the high nitrate level in vegetables, which leads to toxicity. Therefore, NOAEL value is required to be measured following chronic vegetables exposure because of its crucial role in setting human safety standards for chemicals present in our diet. Vegetables can be classified into three categories in terms of their nitrate level: (1) plants with high nitrate concentration (>1000 mg.kg\(^{-1}\)) like lettuce, beetroot, spinach, other green leafy vegetables and herbs; (2) plants with an average nitrate content (50–1000 mg.kg\(^{-1}\)) like potatoes and other vegetables; and (3) plants with low nitrate content (0.5–50 mg.kg\(^{-1}\)) like berries, fruits, cereals, and pod vegetables.\textsuperscript{[23,24]} In 2002, the JECFA reconfirmed an ADI of 3.7 mg.kg\(^{-1}\) for nitrate which is equivalent to 222 mg of nitrate per day for a 60 kg adult.\textsuperscript{[1,25]} While assessing the nitrate intake, sources other than food additives, such as vegetables and drinking water should also be studied.\textsuperscript{[1]}

Based on a study conducted by the European Food Safety Authority (EFSA), recommendations have been given regarding the presence of nitrates in vegetables, including the need to develop methodologies for the analysis of the risks and benefits of foods, study the factors affecting the concentration of nitrates and nitrites and collect data on high levels of nitrates in vegetables in member countries of the committee.\textsuperscript{[26]}

The content of nitrates (NO\(_3\)) in agricultural products is of great importance in the quality of products and greatly affect human health. Due to lack of information about the nitrate level of vegetables in Iran, it seems necessity to measure the level of nitrate in various vegetables available in the local market and assess the health risk to humans upon consumption of the nitrate level based on the acceptable daily intake (ADI) and health risk index (HRI). To this end, samples were collected from Zanjan province located in the northwest of Iran in an eastern longitude of 47°51’−49°00’ and
a northern latitude of 36°20’–37°00’. This province is occupied with a population of about one million persons in an area of 21,773 km². The climate is temperate during the summer and cold and dry during the winter with an average annual rainfall of 330–360 mm, due to which agriculture is the main occupation of those living in Zanjan province.¹²⁷

**Materials and methods**

**Sampling**

Vegetables (332 samples) (listed in Table 1) were purchased randomly from various greengrocery in Zanjan, Iran. The samples were divided into three groups: leafy vegetables, edible and root vegetables and fruit vegetables. All the samples were taken and freshly used for analysis.

**Standard and reagent solutions**

All analytical chemicals including sodium nitrate, Sodium carbonate, Sodium bicarbonate, hydrochloric acid, sulfuric acid and HPLC grade methanol were purchased from Merck (Darmstadt, Germany). Deionized water was used for preparation of the solutions and extraction of the samples.

**Preparation and extraction**

Soil, damaged leaves, inedible and highly dusty parts were removed. However, vegetables were not washed to prevent nitrate reduction resulting from washing. The vegetable samples were crushed and homogenized with a mill. Then, 10 grams of the prepared samples was weighed into a 250 ml flask, to which 200 ml hot water were added, shake for 2–3 min, heated on water bath for 15 min at boiling temperature and further cooled down in a suitable dark environment. The flask was filled to the line mark with deionized water and shake again for 2–3 minutes. The sample was centrifuged at 5000 rpm for 10 minutes. The extract was filtered through filter paper and 20 microliter of the filtrated extract was used for analysis. The content of nitrate in the samples was determined using an Ion chromatograph. The shaker SA31 was purchased from Yamato Scientific Co. Tokyo, Japan. A high-speed refrigerated centrifuge 3–30 K (Sigma CO., Germany) and ultrasonic bath (Daihan Lab Tech CO., Nanyangiu, Korea) was applied to prepare the sample.

**Nitrate determination**

Metrohm 881 Compact IC (Metrohm, Switzerland) equipped with suppressed conductivity detector and Metrohm suppressor module (MSM, 50 mmol/L H₂SO₄) was used. Metrosep A Supp 4 column (250 × 4.6 mm, Serial NO. 7812023) was applied for the separation and the MagIC net (version 2.3) software was used to monitor the system and analyze the data. Mobile phase consisted of sodium hydrogen carbonate (1.0 mmol/L) and sodium carbonate (3.2 mmol/L). Injection volume was 20 µl with a flow rate of 1 mL/min.

| Table 1. Accuracy (Recovery %) and precision (RSD %) of nitrate in indicator vegetable. |
|---------------------------------------------------------------|
| groups                      | Spiked level (mg.kg⁻¹) | Intra-day |                                   | Inter-day |                                   |
|                             |                        | Recovery (%) | Mean ± SD | RSD (%) | Recovery (%) | Mean ± SD | RSD (%) |
| Leafy vegetables            | 1000                   | 96.25 ± 12.8 | 13.4      |          | 88.92 ± 6.8  | 7.9       |
| Tuber and root vegetables   | 170                    | 91.44 ± 2.76 | 3         |          | 99.04 ± 6.3  | 6.4       |
| Fruit vegetables            | 120                    | 87.43 ± 4.3  | 4.9       |          | 97.51 ± 8.6  | 8.8       |

The samples were divided into three main groups, and for each group, the most consumed vegetable was selected as an indicator. Therefore, Leek was selected as an indicator of leafy vegetables group, Potato as an indicator of tuber and root vegetables, and Tomato as an indicator of fruit vegetables (n = 6 in three day).
Method validation

To perform the validation method, BS EN 12014–2: 1997, Foodstuffs was used along with verification. Based on the type of vegetables and the permissible limits, the samples were divided into three main groups, and for each group, the most consumed vegetable was selected as an indicator. It should be noted that a spike was considered for the rest of the vegetables every day. Therefore, Leek was selected as an indicator of leafy vegetables group, Potato as an indicator of tuber and root vegetables, and Tomato as an indicator of fruit vegetables. The performance characteristics of the method including the precision, accuracy, calibration data and linearity of calibration curve, LOD, LOQ, selectivity and real sample analysis were evaluated in present investigation. Two linear calibration curve was constructed using different nitrate standards at the range of 0.1–24 mg.kg$^{-1}$ and 24–96 mg.kg$^{-1}$. Three replicate injections were randomly performed at each level. Twenty five microliter of standard solutions were directly injected into the ion chromatography system.

Precision was determined by six consecutive injections of nitrate at maximum acceptable limit level (1000, 170,120 mg.kg$^{-1}$ for Leek, Potato and Tomato, respectively) in intra-day and inter-day for three days. Recovery was determined through the mean obtained for the six independent replicates of a sample spiked at MPL levels. To precisely measure LOQ and LOD, Shallot was selected as blank sample and then, The LOD and LOQ were estimated by signal-to-noise ratio, 3:1 and 10:1, respectively. Stability of standard solutions in deionized water and spiked samples was conducted. The results confirmed the stability of nitrate in spike samples in refrigerator temperature (4°C) after 5 days. Moreover, no interference chromatogram was observed at the retention time of nitrate in blank and spiked samples, which suggested the selectivity of the proposed method.

Exposure assessment

To assess the risk, daily exposure to nitrate and in fact, daily vegetable consumption and concentration of nitrate in vegetables are needed to be known. Since per capita consumption of vegetables is not available in Iran, questionnaires were designed to estimate the consumption of vegetables. The survey included a sample consisting of 181 adults (aged 15 and over). The daily consumption of the desired vegetables was obtained and then, the daily nitrate exposure (mg.kg$^{-1}$ of body weight/day) was estimated using the following formula. (Equation 1)

$$\frac{\text{Daily vegetable consumption intake(gr)} \times \text{Mean nitrate level in vegetables(mg kg}^{-1})}{\text{Body weight(kg)} \times 1000}$$

(1)

In some cases, where the nitrate level was below the detection limit, half of the detection limit was applied to estimate the exposure assessment.$^{[1,29]}$

Health Risk Index (HRI)

The Health Risk Index (HRI) is dependent on the estimated daily intake (EDI) of the food products and the oral Reference Dose (RfD). The HRI assesses the potential hazard of contaminants to human health depending on daily intake of a given meal. This index was used to estimate the safety thresholds of nitrate exposure, which was derived using the following equation for non-carcinogenic risk assessment of nitrate. The HRI > 1 for nitrate in food products implies the potential hazard of chronic disease for consumers (probably at risk). The oral RfD is the numerical estimate of the daily oral exposure of nitrate to humans, less likely to be harmful during the lifetime. The HRI for nitrate exposure from consumption was measured in previous investigations$^{[4,30]}$ using the following formula:

(Equation 2):
where EDI is Estimated Daily Intake (mg·kg⁻¹ body weight/day), and Rfd denotes the reference dose (mg·kg⁻¹ body weight/day). The oral Rfd of the nitrate nitrogen is 1.6 mg·kg⁻¹ of body weight/day, which is equivalent to 7.09 mg·kg⁻¹ b.w./day of nitrate in accordance with the United States Environmental Protection Agency.  

**Statistical analysis**

SPSS v.16 (SPSS Inc, Chicagow, USA) was used to analyze the data. Descriptive results are presented as the means ± standard deviation (SD), median, interquartile range, maximum, and minimum. As the data were not normally dispersed, the Kruskal–Wallis test was conducted to analyze the relative nitrate content of various food groups. The p values of less than 0.05 were considered to demonstrate statistical significance using the one-way ANOVA test, followed by the post-hoc Tukey test among different types of vegetable.

**Results and discussion**

Despite the numerous benefits of vegetables, contamination with various pollutants can have severe adverse effects on human health. One of the most important contaminants in vegetables is nitrates, which can contaminate vegetables in various ways, for example through the use of nitrate chemical fertilizers. Although the form of N- fertilizers applied for the crop growth is very important, we suppose that analysis of nitrate level in the soil, water and fertilizers is necessary for understanding the NO₃ source. In addition, the irrigation water and native characteristics of the soil are other contamination source of vegetables. Although the incidence of methemoglobinemia is more common in patients faced with high levels of nitrites, studies have shown that intake of large amounts of nitrates is associated with increased incidence of certain cancers and methemoglobinemia. Therefore, regular monitoring and control of nitrates in vegetables and other foods and beverages can prevent various diseases in humans. Thus, valid analysis methods with optimal accuracy and precision and appropriate limit of quantification are needed. Here, ion chromatography method was used to analyze nitrate content in vegetables.

**Performance characteristics of the proposed method**

Our data confirmed no interference at the retention time of nitrate in blank and spiked samples as selectivity of method. The chromatograms of standard, shallot (blank), spiked and real samples are shown in Figure 1. Due to variable levels of nitrate in real samples, two external calibration curves of nitrate standard solutions over the range of 0.1 to 24 mg/L and 24 to 96 mg/L were employed. The calibration curves were linear with good R² (determination coefficient) of 0.9997 and Linear equation \( y = 0.0865x - 0.0123 \) was gained for 0.1 to 24 mg/L standards and \( y = 0.1015x - 0.2469 \) for 24 to 96 mg/L standards solutions with R² of 0.9958. The high value of the regression coefficient indicated a good linearity of the analytical response in proposed samples.

The lowest average recovery and highest relative standard deviation (RSD %) of nitrate were 87.4, and 13.4%, respectively, as described as accuracy and precision in Table 1. RSD values for repeatability and reproducibility varied between 3 to 13.4% to confirm precision of proposed method (Table 1).

The LOD and LOQ of nitrate were determined 0.015 and 0.05 mg/L, respectively, following spiking of blank samples (rhubarb, shallot, Carrot, Tomatoes, Cherry tomatoes and Zucchini). The stability of the column was estimated by calculating the retention time of a standard solution of nitrate following 20 µl injections and the mean of the observed retention time, which was 8.96 min ± 0.24.

The average nitrate content for analyzing of real samples is presented in Table 2. Totally, nitrate was found to be higher than the LOQ in 91% of samples. According to the results, nitrate was detected in
96%, 81% and 81% of the leafy vegetables, the root and tuber vegetables and the fruit vegetables, respectively, in the range of 1.25–1950 mg.kg\(^{-1}\) (Table 2). The lowest and highest level of nitrate was observed in shallot (ND = none detected) and Spinach (1950 mg.kg\(^{-1}\)), respectively (Table 3).

Based on previous studies, organs of vegetable and fruit samples are ordered as follows in terms of nitrate concentration: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed.\(^{[34]}\) The order was similar to what has been found in the present study. The maximum mean concentration of nitrate was observed in Spinach (653 mg.kg\(^{-1}\)) followed by Iceberg lettuce (386.4 mg.kg\(^{-1}\)) and lettuce (366 mg.kg\(^{-1}\)) (Table 2). In none of the samples, the nitrate levels exceeded the maximum allowed limit set by the Iranian National Standard organization and European commission.\(^{[35,36]}\)
The nitrate level was significantly different between the leafy vegetables and other vegetables \((P < .05; \text{Table 1})\). In the present study, the nitrate content of spinach from the leafy vegetables was in the range of 108–1950 mg.kg\(^{-1}\) with a mean level of 653 mg.kg\(^{-1}\) (Table 3), which of course was

Table 2. The nitrate level in different type of vegetables.

| Sample                  | Number | No. Positive (%) | Mean *(mg.kg\(^{-1}\)) | SD  | Minimum (mg.kg\(^{-1}\))* | Maximum (mg.kg\(^{-1}\)) |
|-------------------------|--------|------------------|------------------------|-----|---------------------------|--------------------------|
| leafy vegetables        | 210    | 202(96\(\%\))   | 158                    | 171 | 21                        | 1950                     |
| root and tuber vegetables | 63    | 51 (81\(\%\))    | 32                     | 120 | 1.25                      | 136                      |
| Fruit vegetables        | 59     | 48 (81\(\%\))    | 45                     | 14  | 2.2                       | 148                      |
| Total                   | 332    | 301(91\(\%\))    | 118                    | 209 | 1.25                      | 1950                     |

* Value mentioned in positive samples
much lower than the amount obtained in some studies. Martiny and Restaniz (2003) in a study in Italy reported that nitrate in 23 spinach samples was found to be in the range of 3720 mg.kg$^{-1}$ with the mean level of 1757 $\pm$ 1120 mg.kg$^{-1}$.[37] Tamme et al.2006 analyzed one spinach sample in Estonia with a nitrate level of 2508 mg.kg$^{-1}$.[15]

The mean and maximum level of Nitrate in 100% of the Lettuce samples in present study were found to be 365.7 mg.kg$^{-1}$ and 584.6 mg.kg$^{-1}$, respectively (Table 3). Since, the lettuce core is popular in Iran, core as well as leaf of lettuce was analyzed. Nitrate recovered in the lettuce core samples was in the range of 56.2 to 958.8 mg.kg$^{-1}$. Although the mean nitrate concentration of the lettuce core (inner leaves) was more than that of the leaf (365.7 $\pm$ 182 mg.kg$^{-1}$ vs 547 $\pm$ 276 mg.kg$^{-1}$), there was no significant differences between lettuce and Iceberg lettuce (P = .07).

The mean concentration of nitrate found in this study for lettuce sample (366 mg.kg$^{-1}$) was less than the values obtained by other Iranian studies which reported mean nitrate level of 3585.5 mg.kg$^{-1}$ in 200 lettuce samples.[38] Another study in Iran found a mean of 1072.60 mg.kg$^{-1}$ in 77 lettuce samples.[39] Moreover, the mean level of nitrate in this study was also less than the amount reported by the studies conducted in other countries such as UK (2330 mg.kg$^{-1}$),[40] Estonia (2167 mg.kg$^{-1}$),[15] Korea (2430 mg.kg$^{-1}$),[41] Taiwan (1520 mg.kg$^{-1}$).[42]

The mean and maximum level of nitrate in all samples of Iceberg lettuce were 386 and 529 mg.kg$^{-1}$, respectively. Therefore, no significant difference was observed between the lettuce and Iceberg lettuce (P<0.05). This is the lowest contamination level reported earlier in Iran. In a similar study, mean and maximum level of nitrate in all 180 Iceberg lettuce samples collected from south of Tehran, Iran were 3359.9 mg.kg$^{-1}$ and 3451.4 mg.kg$^{-1}$, respectively.[38]

Celery is a fragrant vegetable that is used in the preparation of various recipes and flavor food. Celery juice is a great source of numerous nutrients such as potassium, calcium, and vitamins A, C and K and many people consume it to maintain their health. High concentration of nitrates in celery was reported to be affected by growing system and nitrogen content in the nutrient solution.[43] In this study, the mean and maximum level of nitrate in all the samples were 147 $\pm$ 73 and 337 mg.kg$^{-1}$, respectively.

Contrary to the data collected from other countries, the mean concentration of nitrate in celery samples (147 mg.kg$^{-1}$) in this study was less than 1496 mg.kg$^{-1}$ in USA, 1610 mg.kg$^{-1}$ in New Zealand,[45] 595 mg.kg$^{-1}$ in Estonia,[15] 124 mg.kg$^{-1}$ in France,[46] and 3600 mg.kg$^{-1}$ in China.[41] In the USA, the mean nitrate content in conventional broccoli, cabbage, and celery was 394, 418, and 1496 mg.kg$^{-1}$, respectively.[44]

The spinach and lettuce as some typical leafy vegetables had higher nitrate content. Here, the amount of contamination with nitrate in a leafy vegetable sample was higher compared with other vegetables. The accumulation of Nitrates in leafy vegetables can be affected by several factors including the type of soil, the density in the field, environmental temperature and humidity, maturity, harvesting time, vegetation period, and source of applied nitrogen.[47] In addition, various parts of vegetables and the age of plant tissue during consumption may influence the nitrate level. Younger and inner leaves contained less nitrate compared with older and outer ones.[48]

Minimum concentration of nitrate was observed in the root and tuber vegetables. The lowest mean nitrate concentration was observed in shallot (none), carrot (23 mg.kg$^{-1}$) and rhubarb (26 mg.kg$^{-1}$). In this group, nitrate was recovered in 51 out of 63 samples (81%). The nitrate amount in all of shallot samples was lower than the detection limit (LOD). The highest concentration of nitrate was observed in potato samples followed by radish and onion with a mean concentration of 37.5, 36.32 and 136.5 mg.kg$^{-1}$, respectively. The maximum level of nitrate in potato, radish and onion was 136.5, 54.2 and 53.9 mg.kg$^{-1}$, respectively.

Potato as one of the root and tuber vegetables is widely consumed all over the world. High consumption of this vegetable can lead to significant amounts of nitrate, and cause health problems for consumers. In this study, nitrate was recovered in all of the samples with a mean of 38 and a maximum level of 137 mg.kg$^{-1}$. The results of this study corresponds
Table 3. The number positive samples mean, median, IQR, minimum and maximum level of nitrate level in vegetables.

| Sample type          | Sample  | No. Positive (%) | Mean ± SD (mg.kg\(^{-1}\)) | Median | IQR | Minimum (mg.kg\(^{-1}\)) | Maximum (mg.kg\(^{-1}\)) | MPL(EU & Iran) (mg.kg\(^{-1}\)) | >MPL (mg.kg\(^{-1}\)) |
|----------------------|---------|------------------|-----------------------------|--------|-----|--------------------------|--------------------------|-------------------------------|------------------|
| Leafy Vegetables     | lettuce | 12 (100%)        | 366 ± 182                   | 393    | 235 | 47                        | 585                      | 1500                          | -                |
|                      | Spinach | 12 (100%)        | 653 ± 711                   | 283    | 1072| 108                       | 1950                     | 2000                          | -                |
|                      | iceberg lettuce | 12 (100%) | 386 ± 133                   | 397    | 111 | 21                        | 529                      | 1500                          | -                |
|                      | Artichoke | 12 (100%) | 45 ± 16                     | 39     | 29  | 28                        | 77                       | 400                           | -                |
|                      | Celery   | 12 (100%)        | 147 ± 73                    | 129    | 78  | 73                        | 337                      | 400                           | -                |
|                      | Rhubarb  | 12 (33.33)       | 26 ± 2                      | 27     | 4   | 23                        | 28                       | 400                           | -                |
|                      | beet leaf | 12 (100%)       | 134 ± 57                    | 113    | 94  | 71                        | 261                      | 1000                          | -                |
|                      | Fenugreek| 12 (100%)        | 31 ± 7                      | 29     | 10  | 23                        | 48                       | 1000                          | -                |
|                      | Cabbage  | 12 (100%)        | 49 ± 48                     | 38     | 14  | 21                        | 200                      | 500                           | -                |
|                      | Tarragon | 11 (100%)        | 37 ± 24                     | 26     | 6   | 23                        | 88                       | 1000                          | -                |
|                      | Parsley  | 12 (100%)        | 64 ± 11                     | 65     | 19  | 50                        | 81                       | 1000                          | -                |
|                      | Spearmint| 12 (100%)        | 67 ± 23                     | 64     | 34  | 39                        | 119                      | 1000                          | -                |
|                      | Basil    | 12 (100%)        | 43 ± 24                     | 33     | 23  | 26                        | 105                      | 1000                          | -                |
|                      | Leek     | 12 (100%)        | 46 ± 13                     | 46     | 20  | 27                        | 67                       | 1000                          | -                |
|                      | watercress | 12 (100%) | 246 ± 67                    | 251    | 119 | 139                       | 336                      | 1000                          | -                |
|                      | Dill     | 12 (100%)        | 97 ± 18                     | 70     | 25  | 63                        | 132                      | 1000                          | -                |
|                      | Cauliflower | 7 (100) | 34 ± 2                      | 34     | 4   | 32                        | 37                       | 500                           | -                |
|                      | Coriander | 12 (100%)      | 141 ± 38                    | 148    | 79  | 91                        | 186                      | 1000                          | -                |
| root and tuber vegetables | Radish | 12 (100%)        | 36 ± 10                     | 38     | 17  | 22                        | 54                       | 500                           | -                |
|                      | Onion    | 12 (100%)        | 32 ± 11                     | 32     | 16  | 21                        | 54                       | 90                            | -                |
|                      | Shallot  | 12 (0)          | ND                          | ND     | ND  | ND                        | ND                       | 500                           | -                |
|                      | Carrot   | 12 (100%)        | 23 ± 1                      | 23     | 1   | ND                        | 24                       | 250                           | -                |
|                      | Potato   | 15 (100%)        | 38 ± 41                     | 45     | 67  | 1.25                      | 137                      | 170                           | -                |
| Fruit vegetables     | Tomatoes | 12 (83.3)        | 31 ± 11                     | 26     | 18  | 21                        | 50                       | 120                           | -                |
|                      | Cherry tomatoes | 12 (75%) | 37 ± 12                     | 37     | 22  | 23                        | 55                       | 120                           | -                |
|                      | Eggplant | 8 (100%)         | 51 ± 21                     | 46     | 30  | 30                        | 91                       | 400                           | -                |
|                      | zucchini | 7 (71.43%)       | 38 ± 27                     | 27     | 37  | 22                        | 87                       | 400                           | -                |
|                      | Cucumber | 12 (100%)        | 72 ± 49                     | 38     | 98  | 2.5                       | 147                      | 90                            | 1 (8.5%)         |
|                      | Bell Pepper | 8 (100%) | 42 ± 44                     | 27     | 13  | 22                        | 150                      | 200                           | -                |

IQR: Interquartile Range; LOQ = 0.05 mg.kg\(^{-1}\); LOD = 0.015 mg.kg\(^{-1}\).
with the amounts found in Poland (about 54 mg.kg\(^{-1}\))\(^{49}\) and Nigeria (35 mg.kg\(^{-1}\)).\(^{50}\) However, the concentration of nitrate in potatoes in the present study was lower than 350 mg.kg\(^{-1}\) in Kermanshah, Iran,\(^{51}\) 520 mg.kg\(^{-1}\) in Tehran, Iran\(^{38}\) and 130 mg.kg\(^{-1}\) in Hamedan, Iran\(^{39}\) and 150 mg.kg\(^{-1}\) in UK.\(^{40}\) 158 mg.kg\(^{-1}\) in Slovenia,\(^{52}\) 94 mg.kg\(^{-1}\) in Estonia\(^{15}\) and 307.19 mg.kg\(^{-1}\) in Bangladesh.\(^{4}\)

As for onion as another root and tuber vegetable, nitrate in 100% of samples was in the range of 21–54 mg.kg\(^{-1}\) with an average of 32 mg.kg\(^{-1}\). None of the samples had nitrate higher than maximum permissible level (MPL) in Iran. The obtained results are in contrast with the result of former study in Iran. Data from the study done in Hamadan reported that 25.37% of 67 samples had a nitrate level higher than MPL. Nitrate concentration was in the range of 10–397 mg.kg\(^{-1}\) with a mean level of 82.25 mg.kg\(^{-1}\).\(^{39}\) In Estonia, nitrate was recovered in onion samples with a mean and maximum contamination level of 55 mg.kg\(^{-1}\) and 92 mg.kg\(^{-1}\), respectively.\(^{15}\)

Tomato is one of the most widely consumed fruit vegetables in Iran, which is both cooked and used raw in many foods, sauces, and salads. In this survey, nitrate was detected in 83% and 58% of tomato and cherry tomato samples with a mean of 31 ± 11 mg.kg\(^{-1}\) and 37 ± 12 mg.kg\(^{-1}\), respectively. Therefore, no significant difference is seen between tomato and cherry tomato in this regard (p > .05). The amount of nitrate found here is higher than the values reported in former Iranian studies by Salehzadeh and colleague (about 95 mg.kg\(^{-1}\))\(^{24}\) and Mehri and colleague (about 111 mg.kg\(^{-1}\)).\(^{39}\)

The nitrate content in cucumber and bell pepper were found in the range of 2.5 to 147 mg.kg\(^{-1}\) and 22 to 150 mg.kg\(^{-1}\) with a mean of 72 ± 49 and 42 ± 44 mg.kg\(^{-1}\), respectively. Nitrate was observed in 83.3% and 100% of cucumber and bell pepper collected from Zanjan, respectively. In addition, our data revealed that the nitrate level in one of cucumber samples was higher than the permissible limit approved by the Iranian national standard organization (INSO), i.e. 90 mg.kg\(^{-1}\). The amount of nitrate found here is higher than the values reported in former Iranian studies by Rezaei and colleague (about 42 mg.kg\(^{-1}\)).\(^{53}\)

Various parameters may affect the nitrate concentration in the afore-mentioned vegetables, among which is climatic condition, so that low light intensity influences nitrate accumulation.\(^{52}\) Intense light can regulate the activity of the nitrate reductase and change the nitrate levels in the plant. Thus, plant tissues contain less nitrate in spring and summer compared with autumn and winter.\(^{54}\)

Given that vegetables are one of the most important and useful items in the human food basket, it is necessary to provide conditions to reduce the amount of nitrate in vegetables. It can be done through controlling the amount of chemical fertilizers used, creating environmental conditions to reduce nitrate accumulation, using genotypes with less nitrate accumulation\(^{55}\) and storing vegetables in the refrigerator for an appropriate period of time.\(^{56}\)

Based on World Health Organization (WHO) and Food and Agriculture Organization (FAO), at least 400 g of fruits and vegetables is recommended to be used per day.\(^{2}\) The studies have indicated that an average adult consumes about 400 g of different vegetables each day. Thus, the average intake of nitrate is 157 mg/day.\(^{57}\) Furthermore, about 11–41% of daily intake of nitrate enters the organism. Scientific Committee on Food (SCF) determined 3.7 mg.kg\(^{-1}\) of body weight/day as acceptable daily intake (ADI) of nitrate in 2002, which is equal to the intake of 222 mg nitrate/day in a 60-kg adult. Several factors affect the amount and type of vegetables consumed in Iranian families. Firstly, consumption of vegetables is time-dependent, meaning that different kinds of vegetables which are eaten raw with the main meal are highly consumed in summer. Eggplant and cucumber are also consumed in this season to a large extent. Secondly, taste causes a person to consume one type of vegetable more than average and not to consume another one at all or less than average. Furthermore, some vegetables have a different place in Iranian food culture, i.e., they are the basic material used in cooking and accordingly, are more consumed. Because of the mentioned factors (taste, time dependence of consumption) and due to the lack of data on the consumption of vegetables in Iran, it was not possible to calculate the exact amount of consumption of vegetables. Therefore, health risk assessments were carried out for each vegetable individually. To that end, vegetables were divided into several categories to estimate the risk level: a) Vegetables consumed almost daily throughout the year.
and highly consumed in each meal, e.g. about 75 gr per day [Potatoes, onions, tomatoes, carrots]; b) Vegetables used in certain seasons, consumed depending on the type of food [eggplant, zucchini, celery, lettuce (50–200 gr), cucumber (100 g) and cabbage and cauliflower (50 gr) and highly-used in each meal] and c) Vegetables occasionally consumed (shallots). Other leafy vegetables that have not been mentioned are consumed up to about 20 gr. per serving.

As shown in Table 3, the daily intake of nitrate through the consumption of all studied vegetables in one day is less than the amount determined by JECFA.[1] HRI greater than one means that the consumers are exposed to a potential health risk. Furthermore, the value of the HRI index is related to the oral reference dose (RID) and the EDI value of the related food products. In this study, HRI in all of the samples was lower than one. However, collection of consumption data from 191 persons in limited groups of community and the low sample size (332 samples) which caused the uncertainty of the results are two limitations in the present study.

The results of the present study, as seen in Table 4, showed that in none of the studied vegetables with the consumption amounts of 20, 75 and 200 gr per day, the amount of EDI was lower than ADI (3.7 mg.kg\(^{-1}\).bw.day\(^{-1}\)), showing that the health risk of consuming vegetables is low. European Food Safety Authority reported that the ADI level for nitrate is 3.7 mg.kg\(^{-1}\).bw.day\(^{-1}\), which is equal to 222 mg per day for an adult weighing 60 kg.[26]

In the study by Gruszecka-kosowska et al.[58] the risk associated with nitrate-containing vegetables was assessed and their nitrate contents were compared based on where the plants were grown. The results showed that the highest amount of nitrate was related to lettuce in both organic and conventional farms. Moreover, nitrate content in tuberous and root vegetables was higher in conventional farms. Total daily nitrate intake in conventional and organic farms as 39.2% of ADI and 28.5% of ADI, respectively, indicating that the health risk of consuming vegetables originating from both types of farms is low.

In another study, the amount of nitrate and nitrite in leafy vegetables and fruits was shown to be higher than that of fruits and cucurbits. It was also found that among all the measured fruits, the highest amount of nitrate was related to eggplant and the lowest was for tomato, and among vegetables, the highest amount was related to mint and the lowest was for savory. It should be noted that in all the evaluated materials, the nitrate levels were lower than the limit set by WHO.[59] Another study conducted by Bahadoran et al., 2016 revealed that the highest nitrate was observed in vegetables. Furthermore, the mean nitrate value in radish, beetroot, tarragon, lettuce, mint and celery were reported to be 6250 mg.kg\(^{-1}\), 4950 mg.kg\(^{-1}\), 4240 mgkg\(^{-1}\), 3650 mg.kg\(^{-1}\), 2800 mg.kg\(^{-1}\) and 2600 mg.kg\(^{-1}\), respectively. The lowest mean nitrate levels were reported in tomato (170 mg.kg\(^{-1}\)), corn (280 mg.kg\(^{-1}\)), garlic (350 mgkg\(^{-1}\)), potato (380 mgkg\(^{-1}\)), green beans (470 mg.kg\(^{-1}\)), and carrot (500 mg.kg\(^{-1}\)).[60]

Tamme et al.[15] found that about 11% of the spinach and fresh lettuce samples had a nitrate level higher than limit set by the European Commission Regulation No. 2006/1881, which is significantly different from the results of our present study. The results of the present study showed that, on average, the nitrate content in leafy vegetables was higher compared with tuber vegetables and fruit vegetables, which may be associated with the lower use of nitrate fertilizers in the cultivation of tuber vegetables and fruit vegetables. It should be noted that in confirmation of these results, a recent study showed that the highest amount of nitrate was observed in leafy vegetables, then in roots and tubers, and next in fruit vegetables.[4] In another study, it was determined that the content of nitrate in leafy vegetables is between 800 to 4300 mg.kg\(^{-1}\).[42] which is much higher than of the amount reported here. It is possible that the way the samples were collected plays a role in this difference, meaning that the samples collected from urban areas have higher nitrate content than those from marginal and rural areas.[61] Nowrouz et al.,2012 found that the average nitrate content in none of the studied vegetables including cabbage, lettuce, spinach, parsley, coriander, dill, leek, fenugreek, tarragon, fumitory and mint (respectively 61, 781, 83, 707, 441, 501, 1702, 684, 805, 772 and 191 mg.kg\(^{-1}\)) exceeded the permissible limits.[62] However, in a study conducted in 2008 in the southern part of Iran (Ahwaz), the amount of nitrates in tomatoes...
Table 4. The daily intake of nitrate and Health Risk Index (HRI) through the consumption of vegetables.

| Groups | Vegetables       | Estimated consumption per serving | Mean Intake of nitrate $b$ | Maximum intake of nitrate $b$ | HRI Mean | HRI Max |
|--------|------------------|-----------------------------------|-----------------------------|--------------------------------|-----------|---------|
| Group a* | Onion            | 75                                | 0.04                        | 0.06                           | 0.005     | 0.009   |
|         | Carrot           | 75                                | 0.03                        | 0.03                           | 0.004     | 0.004   |
|         | Tomato           | 75                                | 0.03                        | 0.06                           | 0.005     | 0.008   |
|         | Potato           | 75                                | 0.04                        | 0.16                           | 0.006     | 0.022   |
|         | Cherry tomato    | 75                                | 0.03                        | 0.06                           | 0.006     | 0.009   |
|         | Iceberg lettuce  | 75                                | 0.45                        | 0.61                           | 0.062     | 0.086   |
| Group b** | Lettuce         | 75                                | 0.42                        | 0.68                           | 0.060     | 0.095   |
|         | beet greens      | 200                               | 0.41                        | 0.80                           | 0.060     | 0.113   |
|         | Rhubarb          | 200                               | 0.03                        | 0.09                           | 0.011     | 0.012   |
|         | Celery           | 200                               | 0.45                        | 1.04                           | 0.063     | 0.146   |
|         | Spinach          | 200                               | 2.01                        | 6.00                           | 0.283     | 0.846   |
|         | Eggplant         | 200                               | 0.16                        | 0.28                           | 0.022     | 0.039   |
|         | Zucchini         | 200                               | 0.12                        | 0.27                           | 0.016     | 0.038   |
|         | cucumber         | 100                               | 0.11                        | 0.22                           | 0.016     | 0.032   |
|         | Cauliflower      | 50                                | 0.03                        | 0.03                           | 0.004     | 0.004   |
|         | Cabbage          | 50                                | 0.04                        | 0.15                           | 0.005     | 0.022   |
|         | Coriander        | 20                                | 0.04                        | 0.06                           | 0.006     | 0.008   |
|         | Dill             | 20                                | 0.03                        | 0.04                           | 0.004     | 0.006   |
|         | Garden cress     | 20                                | 0.08                        | 0.10                           | 0.010     | 0.015   |
|         | leek             | 20                                | 0.01                        | 0.02                           | 0.001     | 0.003   |
|         | basil            | 20                                | 0.01                        | 0.03                           | 0.002     | 0.005   |
|         | spearmint        | 20                                | 0.02                        | 0.04                           | 0.003     | 0.005   |
|         | parsley          | 20                                | 0.02                        | 0.02                           | 0.003     | 0.004   |
|         | tarragon         | 20                                | 0.01                        | 0.03                           | 0.002     | 0.004   |
|         | Fenugreek        | 20                                | 0.01                        | 0.01                           | 0.001     | 0.002   |
|         | radish           | 20                                | 0.02                        | 0.02                           | 0.002     | 0.002   |
|         | shallot          | 20                                | 0.00                        | 0.00                           | 0.000     | 0.000   |
|         | Artichoke        | 20                                | 0.01                        | 0.02                           | 0.002     | 0.003   |
|         | Bell Pepper      | 20                                | 0.01                        | 0.05                           | 0.002     | 0.007   |

a: mg.kg$^{-1}$, b: mg.kg$^{-1}$.w/day. *Intake estimated based on 75 g of each vegetable per day. **Intake estimated based on 50–200 g of each vegetable per day. ***Intake estimated based on 20 g of each vegetable per day.

(1644 mg.kg$^{-1}$), cucumbers (999 mg.kg$^{-1}$), carrots (252 mg.kg$^{-1}$) and potatoes (213.8 mg.kg$^{-1}$) exceeded the permissible limits.$^{[63]}$ In another study, the average nitrate content in Isfahan province of Iran in studied vegetables including bell pepper, tomato, lettuce and mint (respectively 15, 15, 627, 490 mg.kg$^{-1}$) lower than the permissible limits except one lettuce sample (1799 mg.kg$^{-1}$).$^{[64]}$

**Conclusion**

The present study aimed to evaluate nitrate content in the selected vegetables and fruits and assess EDIs. Based on the data, the mean nitrate level in vegetable and fruits was in the range of 23 to 653 mg.kg$^{-1}$. The highest and lowest EDI were observed in spinach and shallot, respectively. However, since EDIs of nitrate from the consumption of vegetables and fruits are less than the ADI level, the amount of nitrates in raw vegetables may be lower than the standard level, which does not cause health problems for consumers. Although the daily intake of nitrate from vegetables in Zanjan, Iran was less than ADI set by WHO, the amount of
nitrate intake from other foods and drinking water was not examined here. Furthermore, the HRI resulting from the consumption of 200gr of spinach per day in sample with maximum nitrate level (1950 mg.kg\(^{-1}\)) was 0.846, which can be considerable after 30–40 years of exposure, especially in vegetarian. Thus, to avoid health problems or chronic diseases caused by nitrate, it is recommended to regularly monitor the use of nitrogen fertilizers and the nitrate content in vegetables.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**Data availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Author contributions**

Samaneh Dezhangah: Methodology, Investigation, Validation, Data curation, writing Draft; Firuzeh Nazari: Methodology, Investigation, Validation, Data curation, writing draft. Validation, writing—original draft. Kourosh Kamali: Investigation, statistical analysis; Mohammad Reza Mehrasebi: Project administration, Supervision, Writing Draft; Mir-Jamal Hosseini: Project administration, Supervision, Methodology, Investigation, Validation, Data curation, Review & editing.

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