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Original Article

Effects of freezing vegetables on nitrate content and their health-related risks

Ivana Kostić Kokić
University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia
ivana.chem@outlook.com (corresponding author)

Tatjana Anđelković
University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia

Danica Bogdanović
University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia

Abstract:

Vegetables are the major source of nitrate entry into the human body. The present study was conducted to investigate the influence of freezing on nitrate content in 15 species of vegetables from three commonly used groups: leafy, root and fruit vegetables. Determination of nitrate ions in sample extracts was performed using ion chromatography. The highest level of nitrate content before freezing was determined in samples of radish, while the lowest content of nitrate in raw vegetables was determined in samples of pepper. In raw samples of leafy celery and cucumber, nitrates were not detected. Results obtained by analyzing samples after freezing showed that this process has a different influence on the investigated samples. Some samples contained a higher level of nitrate after freezing, while others contained less. Part of the investigated vegetables showed an increased health risk if consumed in an amount of 400 g per day.

Key words:
nitrate, vegetables, freezing, health risk, ion chromatography

Apstrakt:

Uticaj zamrzavanja povrća na sadržaj nitrata i njihov zdravstveni rizik

Povrće je glavni izvor unosa nitrata u ljudski organizam. Ovo istraživanje je sprovedeno sa ciljem ispitivanja uticaja zamrzavanja na sadržaj nitrata u 15 vrsta povrća iz tri najčešće korišćene grupe: lisnato, korenasto i voćno povrće. Određivanje nitratnih jona u ekstraktima uzoraka izvršeno je jonskom hromatografijom. Najviši nivo sadržaja nitrata pre zamrzavanja određen je u uzorcima rotkve, dok je najniži sadržaj nitrata u sirovom povrću određen u uzorcima paprike. U sirovim uzorcima lisnatog celera i krastavca nisu detektovani nitrati. Rezultati dobijeni analizom uzoraka nakon zamrzavanja pokazali su da ovaj proces ima različit uticaj na ispitivane uzorke. Pojedini uzorci su sadržali viši nivo nitrata nakon zamrzavanja, dok su drugi sadržali manji. Deo ispitivanog povrća pokazao je povećan zdravstveni rizik ako se konzumira u količini od 400 g dnevno.

Ključne reči:
nitrati, povrće, zamrzavanje, zdravstveni rizik, jonska hromatografija

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Introduction

Vegetables are a rich source of minerals, vitamins, and biologically active compounds and play an important role in human nutrition (Prasad & Chetty, 2008). They are necessary for human wellbeing, and the World Health Organization (WHO) and Food and Agricultural Organization (FAO) have recommended a minimum intake of 400 g of fruits and vegetables per day to prevent chronic diseases (WHO, 2003; Qasemi et al., 2024).

In recent years, rapid population growth, as well as industrialization, have increased need for more food production. This leads to extensive use of chemical fertilizers, which are nitrogen-based. In these conditions, vegetables can accumulate a notable amount of nitrate and nitrite (Kounnoun et al., 2024; Wang et al., 2024). Nitrogen uptake depends on many biological and environmental factors, including the composition of soil, light intensity, air temperature and moisture, duration of growth period, etc. (Bahadoran et al., 2016). Vegetables represent the major source of nitrate intake in the human diet, and it has been estimated that 75–80% of the total daily intake (TDI) comes from vegetables (Chang et al., 2013a and 2013b).

Both inorganic compounds, nitrite (NO_2^-) and nitrate (NO_3^-), are involved in the nitrogen (N) cycle (Kyriacou et al., 2019). These nitrogen forms are present in edible plants produced endogenously as byproducts of the nitrate-nitrite-nitric oxide (NO) metabolic pathway in humans (Pinaffi-Langley et al., 2025). Nitrate can easily be reduced in the mouth and stomach to nitrite, which can react with secondary amines and then form cancerogenic N-nitrosamines. Also, nitrite has a high affinity for haemoglobin in the blood and can combine vigorously to form methemoglobinemia, known as blue baby syndrome (Kiani et al., 2022). Analysis of nitrate in vegetables is certainly necessary to assess the exposure to nitrate for adverse or beneficial effects (Yu et al., 2018). According to the scientific risk assessment on nitrate in vegetables, requested by the European Commission and adopted by the European Food Safety Authority (EFSA), the FAO/WHO Expert Committee on Food Additives set the acceptable daily intake of 3.7 mg nitrate/kg body weight (FAO/WHO, 2003, 2005a, and 2005b).

Numerous methods have been recommended for determining nitrate in fresh vegetables, but three techniques have been validated by international standards: ISO 6635, Ion chromatography (IC) and Cadmium column reduction (Hasheminasab et al., 2025).

The aims of this research were to determine the amount of nitrate in different types of vegetables that are commonly represented in the human diet in Serbia, the effects of the freezing process on nitrate concentration and the assessment of the health risks of nitrates in vegetables.

Materials and Methods

Sampling

This study was performed during the summer in 2022. Vegetables are classified into three groups: root vegetables, leafy, and fruit vegetables. The population consisted of carrot (*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G.Martens), radish (*Raphanus sativus* L.), celery (*Apium graveolens* L.), parsley (*Petroselinum crispum* (Mill.) Fuss), parsnip (*Pastinaca sativa* L.), lettuce (*Lactuca sativa* L.), chard (*Beta vulgaris* L.), spinach (*Spinacia oleracea* L.), parsley leaves, celery leaves, tomato (*Solanum lycopersicum* L.), bell pepper (*Capsicum annuum* L.), blue tomato, zucchini (*Cucurbita pepo* L.), and cucumber (*Cucumis sativus* L.) cultivated on Niš vegetable fields. All samples were purchased on the local Open market.

Chemicals and instruments

All chemicals used (sodium tetraborate acid, potassium hexacyanoferrate (III) for Carrez I solution and zinc acetate dihydrate for Carrez II solution) were purchased from Sigma Aldrich (Germany). All solutions were prepared using ultra-deionized water. Whatman quantitative filter paper (No. 41) and membrane filters with 0.22 μm pore size were purchased from Merck (Germany).

An analytical balance (ABT 100-5M, Kern, Germany) with precision at ±0.00001 g was used to measure chemicals and samples. Ultra-deionized water was obtained using the Smart2Pure system (Thermo Scientific, USA). Nitrate determination in extracts was done using an ion chromatograph (Dionex Aquion, Thermo Scientific, USA).

Sample preparation

Five samples of all the mentioned vegetables were prepared for analysis. At first, non-edible parts were removed, and vegetables were washed with tap water. Then, vegetables were washed with distilled water, followed by deionized water. The vegetables were dried at room temperature for 24 h. One half of each vegetable was put in polypropylene storage bags, separately, and samples were stored in a freezer for 45 days at -20 °C.

A 10 g portion of each vegetable was homogenized by a mixer (Bosh, Germany). From each homogenised sample, three subsamples of 1.0 g were weighed out by analytical balance and placed into a 100 mL glass. Next, 1.25 mL of disodium tetraborate ($Na_2B_4O_7x10H_2O$) solution and 25 mL of hot ultrapure deionized water (80 °C) were added. The beaker was placed into a boiling water bath for 15 min. After this time, the beaker was removed from the water bath and 0.5 mL of Carrez (I) solution was added and the solution was shaken. Then, 0.5 mL of Carrez (II) solution was added, and it was shaken once more. This solution was allowed to cool down to room temperature. After cooling, the solution was transferred into a 25 mL volumetric flask. The beaker was rinsed with ultra deionized water, and the rinse water was transferred to the same volumetric flask. The ultrapure water was added to fill up the volumetric flask. Solutions were filtered through the Whatman No. 41. Then, the obtained filtrates were filtered through a 0.22 μm cellulose filter prior to chromatographic analysis. A dilution with ultrapure water was necessary due to high concentrations of ions. The final filtrate was diluted to one twenty-fifth to match the dilution for analysis on an ion chromatograph. Each of the five filtrate samples was separated into a replication for nitrate analysis.

Instrumentation

The three replications were analysed for nitrate concentrations as follows. Volume of 5 mL of each filtrate sample was placed into the Dionex AS 50 model autosampler vials. Each of the samples was loaded automatically into the ion chromatograph. Separation was achieved using a Dionex IonPac AS22 column (4×250 mm) with the guard column Dionex IonPac AG22 (4×50 mm). The ion chromatograph used a mix of 4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate as an eluent at a flow rate of 1.2 mL/min. The concentration of nitrate anions in samples was detected by the Dionex AERS 500, Carbonate, 4 mm, conductivity detector. Dionex Seven Anion Standard (Product No. 056933) was used for preparing standard solutions. The nitrate concentration values were calculated automatically based on a previously made processing method using Chromeleon 7 software.

Human health risk assessment

Non-cancerogenic risk assessment was performed by the Target Hazard Quotient (THQ) method provided by the US Environmental Protection Agency (EPA) (USEPA, 2001). The estimated daily intake (EDI) was calculated using Equation 1, and then THQ was calculated using Equation 2.

$$EDI = \frac{EF \times ED \times IRF \times C \times \frac{kg}{1000}}{LT \times BW} \quad (1)$$

$$THQ = \frac{EDI}{RFD} \quad (2)$$

Where EF is exposure frequency (365 days/year), ED is exposure duration (70 years), IRF is average daily intake (g/day), C is concentration in vegetable (mg/kg), LT is average length of life (70 years for non-cancerogenic effect), BW is average body weight (70 kg) and RFD is chronic referent dose for contaminant (3.7 mg/kg of body weight per day). According to the report provided by Milešević et al. (2024), the vegetable consumption in Serbia is 312 g/day.

A one-way ANOVA test was used to examine the significance relationship between vegetable nitrate levels in raw and frozen samples.

The difference between nitrate levels obtained from raw and frozen vegetables was compared to a critical value to determine if the difference is significant. The post-hoc test, Tukey's test, was performed, and the test compares the difference between each pair of mean values with appropriate adjustment for the multiple testing. Values of HSD (honest significant difference) for each pair were computed by the Origin© program. Comparing was performed in case $p<0.05$.

Results and discussion

In total, 15 species of vegetables were examined. Vegetables are divided into three categories: leafy, root, and fruit vegetables.

Nitrate concentrations were determined using ion chromatography, based on a previously defined processing method. Calibration curve showed a good correlation coefficient, 0.99899, in the concentration range 0.25 to 10 mg/L (**Fig. 1**).

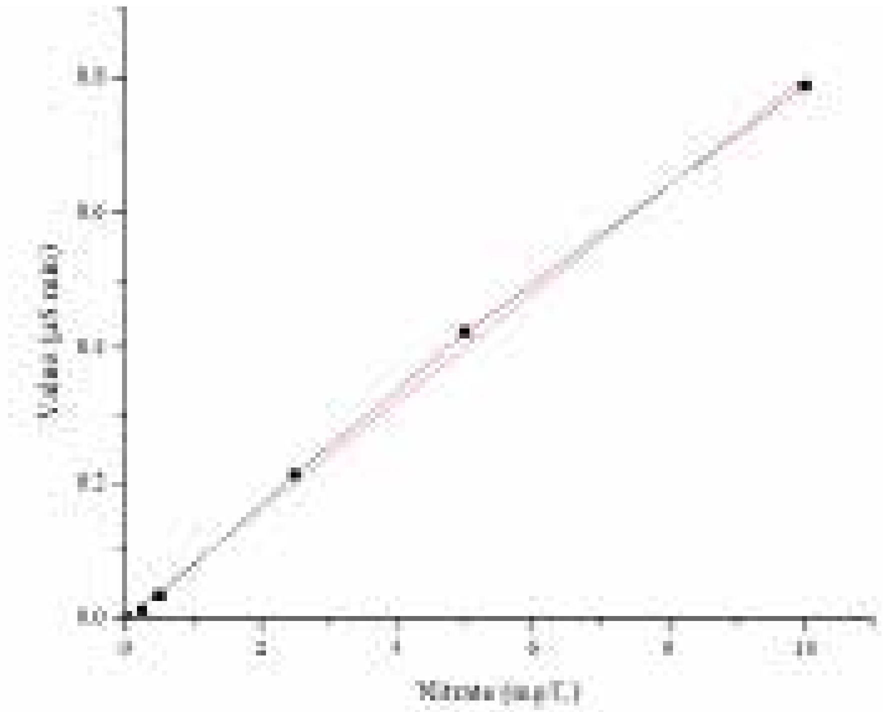

Fig. 1. Calibration curve for nitrate ion at 0.25–10 mg/L concentration range

Figs. 2 and **3** present the chromatograms obtained after analyzing the standard solution and the extract of the radish sample after freezing.

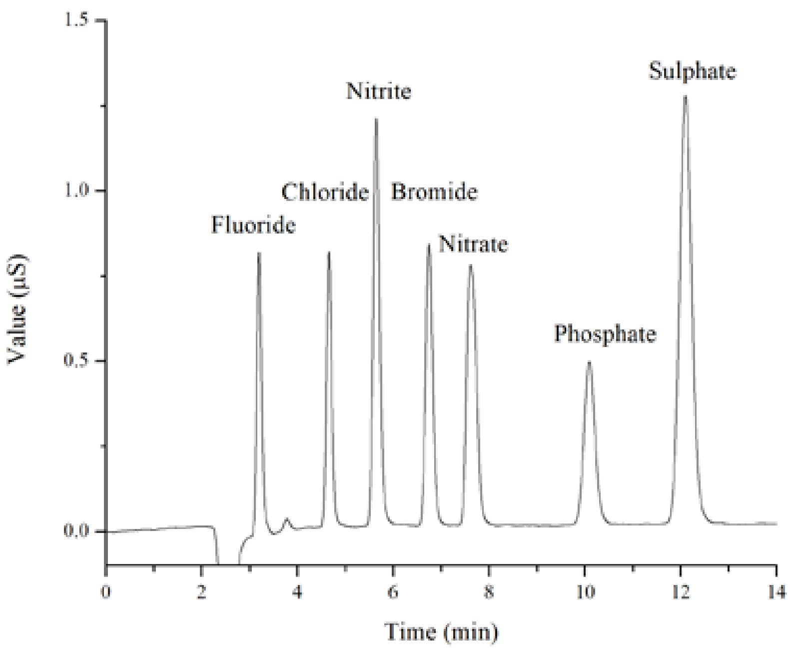

Fig. 2. Chromatograph of standard solution at nitrate concentration 2.5 mg/L

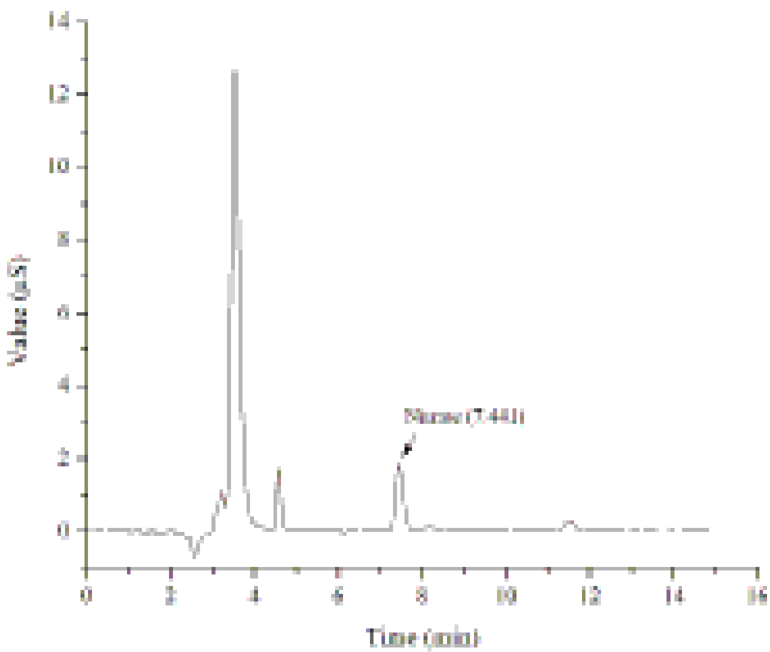

Fig. 3. Chromatogram of frozen radish sample

The present study found that vegetable nitrate concentrations were variable. **Tab. 1** shows the nitrate levels (mg/kg) in all investigated samples of vegetables, raw and frozen. The highest nitrate level in the raw sample of the root vegetables category was in samples of radish with an average of 3406.66 mg/kg, while the lowest nitrate level was in parsley, 315.56 mg/kg. In the same vegetable category, the highest level of nitrates in frozen samples was determined in samples of radish, too (2335.90 mg/kg), as well as the lowest in parsley (126.61 mg/kg). In the category of leafy vegetables, the highest nitrate level in raw samples was determined in lettuce (851.42 mg/kg), as well as in frozen samples (865.52 mg/kg), while in raw samples of celery, nitrates were not detected. The lowest level of nitrate in frozen samples from the category of leafy vegetables was determined in spinach (173.68 mg/kg), while in raw samples, it was detected in samples of parsley (269.37 mg/kg). In the last category, fruit vegetables, the highest level was determined in raw samples of zucchini (2578.33 mg/kg), as well as in frozen samples (1309.76 mg/kg), but in raw samples of cucumber, nitrates were not detected. In frozen samples from fruit vegetables, nitrate level was not detected in tomato, but the lowest determined concentration was in samples of pepper (43.64 mg/kg).

In 10 of 15 vegetables, the nitrate concentrations in frozen samples were lower than in raw samples. Results of Tukey's test showed that there is a significant difference in nitrate level between all raw and frozen samples, except in the samples of lettuce.

The average nitrate level in all raw vegetables was 928.18 mg/kg in raw samples, with the lowest and highest nitrate levels in vegetables being 96.40 mg/kg in pepper and 3406.66 mg/kg in radish. In case of frozen vegetables, the average nitrate level was 592.80 mg/g and the lowest and highest nitrate levels were found in the same vegetables, 43.64 mg/kg in pepper and 2335.90 mg/kg in radish.

The mean level of nitrate in lettuce samples was lower than values reported in literature (Eichholzer & Gutzwiller, 1998; Chung et al., 2011; Ziarati & Arbabi-Bidgoli, 2014; Yu et al., 2018). The amount of nitrate in raw tomato was higher than the results reported in some other studies (Zhou et al., 2000; Ali

Table 1. Nitrate content in each type of investigated vegetable

Vegetable		Nitrate content (mg/kg)	
		Raw	Frozen
Root	Carrot	918.87±4.11[a]	127.57±1.56[b]
	Radish	3406.66±7.41[a]	2335.90±8.70[b]
	Celery	421.56±14.52[a]	831.78±3.39[b]
	Parsley	315.56±5.73[a]	126.61±2.83[b]
	Parsnip	887.26±1.50[a]	0[b]
Leafy	Lettuce	851.42±4.70[a]	865.52±8.89[a]
	Chard	647.53±6.23[a]	266.64±2.06[b]
	Spinach	468.29±6.35[a]	173.68±3.09[b]
	Parsley	269.37±3.28[a]	258.24±1.09[b]
	Celery	0[a]	274.93±2.26[b]
Fruit	Tomato	579.74±2.28[a]	0[b]
	Pepper	96.40±3.20[a]	43.64±2.08[b]
	Blue tomato	625.39±4.34[a]	838.19±3.33[b]
	Zucchini	2578.33±7.94[a]	1309.76±3.49[b]
	Cucumber	0[a]	253.89±5.69[b]

Values with the same letter (**a-b**) within a row are not statistically significantly different at the *p*<0.05 level

et al., 2021). In another study in Mashhad (Zendehbad et al., 2022) reported mean nitrate levels in carrot and cucumber, 355.88 and 221.36 mg/kg, respectively, and these levels were lower for carrot and higher for cucumber. In a study by Bahadoran et al. (2016), nitrate concentrations in radish and celery were significantly higher than concentrations determined in our study (6250 mg/kg and 2610 mg/kg, respectively). Also, investigation of nitrate concentration in samples of chard showed that the obtained results are lower compared to some studies which reported 1690 and 1728 mg/kg (EFSA, 2008; Roila et al., 2018) and for parsley 526 mg/kg (Salehzadeh et al., 2020).

The average amount of any kind of vegetable in the Serbian food basket is not clear. According to data in the literature, 58 g/day of leafy vegetables and 68 g/day of some fruit vegetables, and 39 g/day of root vegetables are consumed from some household baskets (Salehzadeh et al., 2020). Nitrate risks in case of consuming 58, 68, and 39 g/day are presented in **Tab. 2** and **Tab. 3**.

Tab. 2 reveals that the highest health

Table 2. Health risk of nitrates in raw vegetables based on the food basket

Vegetable	EDI for raw vegetables	Health risk (THQ)
Carrot	0.5119	0.1403
Radish	1.8980	0.5200
Celery	0.2349	0.0643
Parsley	0.1758	0.0482
Parsnip	0.4943	0.1354
Lettuce	0.7055	0.1933
Chard	0.5365	0.1470
Spinach	0.3880	0.1063
Parsley	0.2232	0.0611
Celery	0.0000	0.0000
Tomato	0.5632	0.1543
Pepper	0.0936	0.0257
Blue tomato	0.6075	0.1664
Zucchini	2.5047	0.6862
Cucumber	0.0000	0.0000

Table 3. Health risk of nitrates in frozen vegetables based on the food basket

Vegetable	EDI for frozen vegetables	Health risk (THQ)
Carrot	0.0711	0.0195
Radish	1.3014	0.3566
Celery	0.4634	0.1270
Parsley	0.0705	0.0193
Parsnip	0.0000	0.0000
Lettuce	0.7171	0.1965
Chard	0.2209	0.0605
Spinach	0.1439	0.0394
Parsley	0.2140	0.0586
Celery	0.2278	0.0624
Tomato	0.0000	0.0000
Pepper	0.0424	0.0116
Blue tomato	0.8142	0.2231
Zucchini	1.2723	0.3486
Cucumber	0.2466	0.0676

risk in raw vegetables can be ascribed to zucchini at 0.6862, and the lowest health risk in pepper at 0.0257, excluding leaf celery and cucumber in which samples nitrates were not detected in raw samples. Also, considering the data from **Tab. 3**, the highest health risk in frozen vegetables can be ascribed to radish at 0.3566. The lowest health risk in frozen samples was obtained for pepper, as in raw vegetables, at 0.0116. In frozen samples, nitrates were not detected in tomato and parsnip. The obtained results showed that health risk values for most of the investigated samples are far below the maximum allowed value (THQ<1).

But the much higher nitrate risk of consuming vegetables is in the case of the consumption of 400 g/day, which results are presented in **Tab. 4** and **Tab. 5**. In some cases, the health risk is very close to the maximum permissible health risk (raw tomato), while in some cases it is higher than 1. Increased health risk, in case of consuming 400 g/day, was obtained for the following raw samples: carrot, radish, parsnip, lettuce, chard, and zucchini, while in the group of frozen samples, those were: radish, root celery, lettuce, blue tomato, and zucchini. In this study, health risk regarding nitrates in vegetables decreases in the following order: root vegetables, fruit vegetables, and leafy vegetables, respectively.

Table 4. Health risk of nitrates in raw vegetables in accordance with the WHO standard (400 g of vegetable daily intake)

Vegetable	EDI for raw vegetables (400 g)	Health risk (THQ)
Carrot	5.2507	1.4385
Radish	19.4666	5.3333
Celery	2.4089	0.6600
Parsley	1.8032	0.4940
Parsnip	5.0701	1.3891
Lettuce	4.8653	1.3329
Chard	3.7002	1.0137
Spinach	2.6759	0.7331
Parsley	1.5393	0.4217
Celery	0.0000	0.0000
Tomato	3.3128	0.9076
Pepper	0.5509	0.1509
Blue tomato	3.5737	0.9791
Zucchini	14.7333	4.0365
Cucumber	0.0000	0.0000

Conclusion

In this study, nitrate concentration in vegetables and the effect of freezing on nitrate levels were investigated. Also, the health risk assessment was done. The obtained results indicate that the average nitrate concentration in root vegetables is the highest, then in fruit vegetables, and the lowest nitrate concentration is determined in leafy vegetables. The results of this study indicate that the freezing process reduces the nitrate amount in all investigated root vegetables except celery. In a group of leafy vegetables, nitrate concentration after freezing and storing was higher in samples of lettuce and celery, while in a group of fruit vegetables, it was the case in samples of blue tomato and cucumber. The health risk is directly related to the nitrate concentrations in vegetables. Therefore, the highest average health risk showed root vegetables, then fruit vegetables, and at the end, the lowest health risk showed leafy vegetables. It should be emphasized that consuming vegetables is only one way to intake nitrate. It means that if the value of health risk is lower than 1, it cannot alone indicate a healthy level of nitrate intake. Other nitrate sources, such as other foodstuffs and drinking water, should be considered to determine the nitrate health risk. Therefore, the

Table 5. Health risk of nitrates in frozen vegetables in accordance with the WHO standard (400 g of vegetable daily intake)

Vegetable	EDI for frozen vegetables (400 g)	Health risk (THQ)
Carrot	0.7290	0.1997
Radish	13.3480	3.6570
Celery	4.7530	1.3022
Parsley	0.7235	0.1982
Parsnip	0.0000	0.0000
Lettuce	4.9458	1.3550
Chard	1.5237	0.4174
Spinach	0.9925	0.2719
Parsley	1.4757	0.4043
Celery	1.5710	0.4304
Tomato	0.0000	0.0000
Pepper	0.2494	0.0683
Blue tomato	4.7897	1.3122
Zucchini	7.4843	2.0505
Cucumber	1.4508	0.3975

control of possible contamination sources and the minimization of vegetable nitrate concentration are recommended.

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References

Ali, R.A., Muhammad, K.A., & Qadir, O.K. (2021). A survey of nitrate and nitrite contents in vegetables to assess the potential health risks in Kurdistan, Iraq. *IOP Conference Series: Earth and Environmental Science*, *910*(1), 012065. https://doi.org/10.1088/1755-1315/910/1/012065

Bahadoran, Z., Mirmiran, P., Jeddi, S., Azizi, F., Ghasemi, A., & Hadaegh, F. (2016). Nitrate and nitrite content of vegetables, fruits, grains, legumes, dairy products, meats and processed meats. *Journal of Food Composition and Analysis*, *51*, 93–105. https://doi.org/10.1016/j.jfca.2016.06.006

Chang, A.C., Yang, T.Y., & Riskowski, G.L. (2013a). Ascorbic acid, nitrate, and nitrite concentration relationship to the 24 hour light/dark cycle for spinach grown in different conditions. *Food Chemistry*, *138*(1), 382–388. https://doi.org/10.1016/j.foodchem.2012.10.036

Chang, A.C., Yang, T.Y., & Riskowski, G.L. (2013b). Changes in nitrate and nitrite concentrations over 24 h for sweet basil and scallions. *Food Chemistry*, *136*(2), 955–960. https://doi.org/10.1016/j.foodchem.2012.08.084

Chung, S., Tran, J., Tong, K., Chen, M., Xiao, Y., Ho, Y.Y., & Chan, C. (2011). Nitrate and nitrite levels in commonly consumed vegetables in Hong Kong. *Food Additives and Contaminants: Part B*, *4*, 34–41. https://doi.org/10.1080/19393210.2011.557784

EFSA (European Food Safety Authority). 2008. *Nitrate in vegetables Scientific Opinion of the Panel on Contaminants in the Food chain*. Retrieved from Nitrate in vegetables - Scientific Opinion of the Panel on Contaminants in the Food chain

Eichholzer, M. & Gutzwiller, F. (1998). Dietary nitrates, nitrites, and N-nitroso compounds and cancer risk: a review of the epidemiologic evidence. *Nutritional Reviews*, *56*(4 Pt. 1), 95–105. https://doi.org/10.1111/j.1753-4887.1998.tb01721.x

Hasheminasab, K.S., Cheraghi, M., Marzi, M., Shahbazi, K., Beheshti, M., & Tavanamehr, A. (2025). Evaluation of nitrate determination techniques in fresh vegetables: A comparison of spectrophotometric and chromatography methods. *Journal of Agriculture and Food Research*, *19*, 101638. https://doi.org/10.1016/j.jafr.2025.101638

Kiani, A., Sharafi, K., Omer, A. K., Matin, B. K., Davoodi, R., Mansouri, B., Sharafi, H., Soleimani, H., Massahi, T., & Ahmadi, E. (2022). Accumulation and human health risk assessment of nitrate in vegetables irrigated with different irrigation water sources-transfer evaluation of nitrate from soil to vegetables. *Environmental Research*, *205*, 112527. https://doi.org/10.1016/j.envres.2021.112527

Kounnoun, A., Kounnoun, K., Cheyadmi, S., El Baaboua, A., Alahlah, N., & El Maadoudi, M. (2024). Development and validation of a UPLC-PDA method for simultaneous nitrate and nitrite determination in vegetable, fruit and meat-based baby foods. *Journal of Food Composition and Analysis*, *130*, 106177. https://doi.org/10.1016/j.jfca.2024.106177

Kyriacou, M., Soteriou, G.A., Colla, G., & Rouphael, Y. (2019). The occurrence of nitrate and nitrite in Mediterranean fresh salad vegetables and its modulation by preharvest practices and postharvest conditions. *Food Chemistry*, *285*, 468–477. https://doi.org/10.1016/j.foodchem.2019.02.001

Milešević, J., Zeković, M., Šarac, I., Knez, M., Krga, I., Takić, M., Martačić, J.D., Stevanović, V., Vidović, N., Ranković, S., Kadvan, A., & Gurinović, M. (2025). Energy and Macronutrient Dietary Intakes of Serbian Adults 18–64 Years Old: EFSA EU Menu Food Consumption Survey in Serbia (2017–2022). *Foods*, *14*, 1228. https://doi.org/10.3390/foods14071228

Pinaffi-Langley, A.C.C., Nguyen, H.V.M., Whitehead, D., Roseland, J.M., Heydorn, K.C., Wu, X., Pehrsson, P.R., Hays, F.A., & Hord, N.G. (2025). Nitrate and nitrite quantification in U.S. vegetable-based baby foods and infant formula via ozone chemiluminescence. *Journal of Food Composition and Analysis*, *137*(A), 106902. https://doi.org/10.1016/j.jfca.2024.106902.

Prasad, S. & Chetty, A.A. (2008). Nitrate-N determination in leafy vegetables: Study of the effects of cooking and freezing. *Food Chemistry*, *106*(2), 772–780. https://doi.org/10.1016/j.foodchem.2007.06.005

Qasemi, M., Ghorbani, M., Salehi, R., Attari, S.M., Afsharnia, M., Dehghani, M.H., Farhang, M., Zarei, A., Gholinejad, A., & Zarei, A. (2024). Human health risk associated with nitrates in some vegetables: A case study in Gonabad. *Food Chemistry Advances*, *4*, 100721. https://doi.org/10.1016/j.focha.2024.100721

Roila, R., Branciari, R., Staccini, B., Ranucci, D., Miraglia, D., Altissimo, M.S., Mercuri, M.L., & Haouet, N.M. (2018). Contribution of vegetables and cured meat to dietary nitrate and nitrite intake in Italian population: Safe level for cured meat and controversial role of vegetables. *Italian Journal of Food Safety*, *7*, 7692. https://doi.org/10.4081/ijfs.2018.7692

Salehzadeh, H., Maleki, A., Rezaee, R., Shahmoradi, B., & Ponnet, K. (2020). The nitrate content of fresh and cooked vegetables and their health-related risks. *PLoS ONE*, *15*(1), e0227551. https://doi.org/10.1371/journal.pone.0227551

USEPA (US Environmental Protection Agency). 2001. *Risk Assessment Guidance for Superfund-Part A, Process for Conducting Probabilistic Risk Assessment (Vol. III). EPA 540-R-02-002Washington, DC: Office of Emergency and Remedial Response*. Retrieved from https://www.epa.gov/risk/risk-assessment-guidance-superfund-rags-volume-iii-part

Wang, M., Liu, Y., Cai, Y., Song, Y., Yin, Y., & Gong, L. (2024). Inhibition of nitrate accumulation in vegetable by *Chroococcus* sp. and related mechanisms, *Rhizosphere*, *31*, 100934. https://doi.org/10.1016/j.rhisph.2024.100934.

WHO (World Health Organisation). 2003. *Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation*. Retrieved from https://www.who.int/publications/i/item/924120916X

WHO (World Health Organisation). 2005a. *Dietary intake of fruit and vegetables and management of body weight (prepared by Tohill, B.C.)*. Retrieved from https://iris.who.int/handle/10665/43145

WHO (World Health Organisation). 2005b. *Dietary intake of fruit and vegetables and risk of diabetes, mellitus and cardiovascular diseases (prepared by Bazzano, L.C.)*. Retrieved from https://iris.who.int/handle/10665/43146

Yu, T.H., Hsieh, S.P., Su, C.M., Huang, F.J., Hung, C.C., & Yiin, L.M. (2018). Analysis of Leafy Vegetable Nitrate Using a Modified Spectrometric Method. *International Journal of Analytical Chemistry*, 6285867. https://doi.org/10.1155/2018/6285867

Zendehbad, M., Mostaghelchi, M., Mojganfar, M., Cepuder, P., & Loiskandl, W. (2022). Nitrate in groundwater and agricultural products: Intake and risk assessment in northeastern Iran. *Environmental Science and Pollution Research*, *29*(52), 78603–78619. https://doi.org/ 10.1007/s11356-022-20831-9

Zhou, Z.Y., Wang, M.J., & Wang, J.S. (2000). Nitrate and nitrite contamination in vegetables in China. *Food Reviews International*, *16*(1), 61–76. https://doi.org/10.1081/FRI-100100282

Ziarati, P. & Arbabi Bidgoli, S. (2014). Investigation of cooking method on nitrate and nitrite contents in crops and vegetables and assess the associated health risk. *International Journal of Plant Animal and Environmental Sciences*, *4*(2), 46–52.