Research article

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Determination of nitrate and nitrite in agricultural crops distributed in northeast of Iran

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Abstract

Background and objective: Intake of nitrate and nitrite through food products may lead to toxicity and carcinogenicity in human. Agricultural crops which have a great share in daily basket of people are prone to nitrate accumulation. Therefore, we aimed to determine the concentration of nitrate and nitrite in some agricultural crops distributed in Mashhad city (northeast of Iran).

Materials and methods: Green vegetables, lettuce, tomato, potato, onion, carrot, watermelon, melon, rice, and wheat were analyzed in our study. Green vegetables were included to parsley, leek, and basil. The samples were collected from April 2017 to March 2020 from local market of Mashhad. Molecular absorption spectrophotometric method was used for determination of nitrite and nitrate concentration in the samples.

Results and conclusion: Due to the low concentration of nitrite in the products, total nitrite (sum of nitrite and nitrate concentration) is reported in the results. The highest concentration of total nitrite was detected in lettuce (1078 ±360.97 mg/kg) and green vegetables (860.53 ±323.43 mg/kg), respectively. Carrot (171.60 ±98.44 mg/kg) and potato (160.66 ±73.74 mg/kg) were moderately contaminated by the pollutants. The lowest contamination was observed in melon (72.40 ±56.41 mg/kg), wheat (24.65 ±28.39 mg/kg), watermelon (22.22 ±25.60 mg/kg), rice (20.81 ±19.98 mg/kg), tomato (11.75 ±10.79 mg/kg), and onion (9.29 ±11.90 mg/kg), respectively. Compared to the maximum permitted level of total nitrite determined by the Iranian regulation, approximately 34% of green vegetables and 8% of lettuce were rejected. In addition, 48% of potato samples were out of range of total nitrite. As a conclusion, strict control and monitoring of the agricultural crops is required to ensure safety of the products delivered to the consumers.

Keywords: Agricultural crops, maximum permitted limit, nitrate, nitrite, spectrophotometric method

1. Introduction

In recent decades, monitoring of toxic chemicals residues in agricultural crops has been important due to the growing rate of human diseases worldwide. Nitrite and nitrate are of concern in this regard because they commonly enter the

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agricultural crops from the environment. Indeed, excessive use of low-cost nitrogen fertilizers may lead to accumulation of nitrate in edible portions of the crops. Fruits and vegetables have positive effects in prevention of coronary heart disease. However, high concentrations of toxic chemicals in the products are strongly correlated with increased risk of cancers in adults and methemoglobinemia in young children. Vegetables and drinking water are the major sources of nitrite and nitrate intake (approximately 80%), and the other sources provide less amounts in the diet [1,2].

Contribution of drinking water and vegetables in nitrite/nitrate intake is more than cured meats sometimes. Although, it has been shown that diets rich in fruits and vegetables have low contribution in development of cancers possibly due to their high antioxidant level [3]. However, reduction and/or elimination of toxic compounds from farm to fork is important favored to administration of healthy products by the consumers.

Nitrate is not as toxic as nitrite, but it is converted to nitrite. Approximately, 5% of ingested nitrate is converted to toxic nitrite through microbial reduction mechanisms in the gastrointestinal tract [4]. Nitrite reaction with oxyhemoglobin (oxyHb) produces methemoglobin (metHb) and nitrate. The degenerative mechanisms are propagated by the activity of protein-free radicals, ferryl hemoglobin, nitrogen dioxide (NO_2), and hydrogen peroxide (H_2O_2) through autocatalytic free radical chain reactions [5-7]. Formation of met-Hb, known as methemoglobinemia, and its further effects is considered as the acute toxicity of nitrite. MetHb is not able to bind oxygen and results in hypoxia and cell death. On the other hand, the adverse effect of nitrite become more problematic when vitamin C intake is low or there is low gastric acidity. Formation of N-nitroso compounds is associated with chronic effects of nitrite. N-nitroso compounds have shown carcinogenic effects in more than 40 animal species [8]. Vitamin C blocks the routes of N-nitroso compounds formation *in vivo* and *in vitro*. Ascorbic acid reacts with nitrite and produces dehydroascorbic acid and nitric oxide. Nitric oxide is responsible for the pink color of nitrosomyoglobin in cured meats. Reactivity of ascorbate anion with nitrite is 230 times faster than ascorbic acid; therefore, efficiency of ascorbic acid at moderate acidity (pH 3-5) is higher than high acidity (pH 1-3) [9].

Daily administration of agricultural crops especially fruits and vegetables are strongly recommended by the dietitians due to their high antioxidant level especially vitamin C. As mentioned above, these products accumulate the toxic chemical compounds available in the environment in their body. Therefore, concentration of nitrite/nitrate in the agricultural crops, that are largely consumed by people every day, is of concern. It is important to quantify these compounds precisely in the food products, to find out the arisen risk in the consumers. Thus, aim of the present study was to evaluate the amounts of nitrate and nitrite present in the agricultural crops distributed in local market of Mashhad city (northeast of Iran).

2. Materials and methods

2.1. Materials

Ten types of agricultural crops including green vegetables (parsley, leek, and basil), lettuce, tomato, potato, onion, carrot, watermelon, melon, rice, and wheat were purchased from local markets of Mashhad, Iran. All the chemicals and reagents used for analysis including borax, potassium ferrocyanide, zinc acetate, sulphanilamide, naphthylethylenediamine, and cadmium were of analytical grade and prepared from Merck company (Germany).

2.2. Sample preparation

Spectrophotometry method was used to analyze the products according to ISO 6635 with some modifications [10,11]. Wheat and rice were milled by electric miller. Green vegetables, lettuce, tomato, and carrot were grinded by electric blender. Rinds of melon and watermelon and skin of potato and onion were removed by knife before grinding. About 10 g of the homogenized samples were used for analysis.

2.3. Nitrate and nitrite analysis

At first, 10 g of each sample was moved to 200-ml volumetric flask. Then, 150 ml distilled water at 70 ºC for rice and wheat and at 90 ºC for the other samples was added and the mixture was stirred, followed by addition of 5 ml saturated solution of borax (5 g/100). All the flasks were placed in a water bath at 70 ºC for rice and wheat and at 90 ºC for the others for 30 min. In the next step, 2 ml potassium ferrocyanide (10 g/100 ml) and 2 ml zinc acetate (21.9 g/100 ml containing 3 ml acetic acid) solutions were added to each flask and were left to cool down. The flasks were made up to 200 ml by distilled water and mixed. Then, their content was passed through a nitrate-free filter paper (solution A). After that, 20 ml of solution A was transferred to 50-ml volumetric flask, followed by addition of 3 ml hydrochloric acid (3.5% v/v), 5 ml sulphanilamide (0.2% w/v), and 1 ml naphthylethylenediamine (0.1 g/l) solutions. The flasks were made up to 50 ml by distilled water. Then, the content was mixed and used for nitrite determination (solution B). All the steps were done for each sample separately.

For determination of nitrate, 10 ml of solution A was reduced by cadmium in 50-ml conical flask in the presence of buffer solution (NH_4Cl, pH 9.6) under stirring for 10 min. The mixture was filtered further by using nitrate-free filter paper in 50-ml volumetric flask. The volume was made up to 50 ml with distilled water (solution C). Then, 5-10 ml of solution C was transferred to 50-ml volumetric flask, followed by addition of the reagents used for preparation of solution B. The final mixture was used for nitrate determination. All the steps were done for each sample separately.

Nitrite and nitrate concentrations were determined by Cecil spectrophotometer (CE 1021, UK) at 538 nm. Calibration curves of nitrite and nitrate were prepared by dissolving 3 g sodium nitrite or potassium nitrate in 1000 ml distilled water as stock solution. Standard solutions at concentrations of 0.5, 1, 2, 2.5, and 3 mg/l were prepared from the stock solution. A fitted regression line (R^2=0.99) was used to calculate the concentration of nitrite and nitrate in the samples.

3. Results and discussion

Determination of nitrite and nitrate by Cd/spectrophotometric method is a reliable approach and provides valid results [12]. Therefore, because of its availability and convenience, we used Cd/spectrophotometry to evaluate nitrite and nitrate concentration in the agricultural crops.

Accumulation of nitrate in agricultural crops is associated with type of product, genetic, environmental factors (such as relative humidity and temperature), and agricultural factors (such as type of herbicide and availability of nutrients) [4]. For example, increased supply of nitrogen by the fertilizers increases nitrate accumulation in the vegetables or insufficient sulfur supply results in enhanced nitrate absorption by mung bean [13]. In addition, there is an indirect correlation between the size of product and nitrate/nitrite concentration, so that the larger vegetables accumulate lower pollutants [14]. It might be due to the conversion of nitrate to protein by the leaves during plant maturation [15].

Nitrite concentration in all of our samples was negligible that is consistent with the results of other studies [16-18]. Therefore, amount of total nitrite (sum of nitrite and nitrate, as described in ISIRI no. 16596) is reported in the current work. According to Table 1, total nitrite concentration was varied among the products. Lettuce (1078.89 ±360.97 mg/kg) and green vegetables (860 ±323.43 mg/kg) showed the highest concentration, while carrot (171.60 ±98.44 mg/kg) and potato (160.66 ±73.74 mg/kg) were contaminated by a moderate concentration. In comparison, onion (9.29 ±11.90 mg/kg), tomato (11.75 ±10.79 mg/kg), rice (20.81 ±19.98 mg/kg), watermelon (22.22 ±25.60 mg/kg), wheat (24.65 ±28.39 mg/kg), and melon (72.40 ±56.41 mg/kg) showed the lowest concentration of total nitrite. The highest concentration of total nitrite in lettuce and green vegetables was in agreement to the results reported by other researchers [18-20].

Table 1- Total nitrite concentration in the agricultural crops distributed in local markets of Mashhad (Iran)

Crops	Total nitrite (mg/kg)	Nitrite (mg/kg)	Minimum concentration of total nitrite (mg/kg)	Maximum concentration of total nitrite (mg/kg)	Rejected samples (%)	National regulation (mg/kg)
Green vegetables	860.53 ±323.43[a]	ND	219.21	1657.11	34	1000
Lettuce	1078.89 ±360.97	0.1-8.6	441.47	2185.64	8	1500
Tomato	11.75 ±10.79	ND	0.25	44.44	0	120
Potato	160.66 ±73.74	0.03-0.90	55.20	290.73	48	170
Onion	9.29 ±11.90	ND	0.35	47.67	0	90
Carrot	171.60 ±98.44	ND	42.32	496.78	20	250
Watermelon	22.22 ±25.60	ND	0.45	107.95	8	60
Melon	72.40 ±56.41	ND	3.00	277.92	36	90
Rice	20.81 ±19.98	ND	0.39	90.23	4	50
Wheat	24.65 ±28.39	ND	0.48	140.15	10	50

*The results are presented as means ±SD.

Table 2 summarizes the results of nitrite and nitrate content in fruits and vegetables in different countries. As seen in the table, in most of the studies lettuce or green vegetables (such as leek, spinach, etc.) had the highest nitrate concentration. Importantly, effect of climatic in concentration of nitrate was reported for some plants such as spinach, cabbage, and eggplant [21].

Table 2- Summary of other studies about determination of nitrite and nitrate concentration in agricultural crops

Crops	Result	Reference
108 types of fruits and vegetables	The highest and the lowest nitrate concentration was observed in leafy and non-leafy vegetables, respectively.	[23]
924 samples of 14 agricultural crops including potato, lettuce, apples, carrot, silage maize, cabbage, grapes, peaches, string beans, cereals, pears, cucumbers, strawberries, and tomato	The highest nitrate concentration was observed in lettuce, cabbage, string beans, and carrot. Moderate contents were observed in potato, silage maize, strawberries, cucumber, and cereals. Fruits (grape, peach, apple, and pear) and tomato had low concentration of nitrate (< 6 mg/kg). Except for cereals (8.9 mg/kg), apple (1.5 mg/kg), potato (1.2 mg/kg), and pear (1 mg/kg), concentration of nitrite did not exceed 0.5 mg/kg.	[18]
Fermented and acidified vegetables (46 commercially available products)	Nitrate contents ranged from 122 mg/100 g for kimchi to undetectable levels in acidified Brussels sprouts. Nitrite concentration was low (<1.5 mg/100 g) and detected in four acidified (pickled green beans, red cabbage, pickled beets, and pickled mushrooms) and two fermented products (Greek olives and kimchi).	[24]
Lettuce, leek, potato, beetroot, Chinese cabbage, and white cabbage	Nitrate content was the highest in lettuce followed by beetroot, Chinese cabbage, fresh spinach, leek, frozen spinach, white cabbage, and potatoes. Seasonal variation was seen in the vegetables. Nitrite content was generally low, however high content was observed in spinach due to inappropriate storage.	[20]

1872 samples of 87 food items	Highly contaminated vegetables by nitrate were included to radish (6.3 mg/g), beetroot (4.9 mg/g), tarragon (4.2 mg/g), lettuce (3.6 mg/g), mint (2.8 mg/g), and celery (2.6 mg/g).	[2]
Fresh and frozen vegetables; fresh and frozen fruits such as different kind of apple, strawberry, currant, raspberry	High concentration of nitrate was observed in lettuce, frozen spinach, fennel, radishes, parsley (>1000 mg/kg). The highest level (> 3500 mg/kg) was found in lettuce. Nitrite concentrations varied from zero to decimal parts of mg/kg.	[16]
Potato, cabbage, Chinese cabbage, scallion (shallot), celery, cucumber, tomato, eggplant, and wax gourd	Generally, nitrite content was low. Average nitrate and nitrite intake from these resources was calculated as 422.8 and 0.68 mg/day, respectively.	[25]
Leafy vegetables including Swiss chard, sea beet, spinach, and cabbage, cauliflower, fruity vegetables such as eggplant, and vegetable marrow grown with organic fertilizers	Leafy vegetables had much higher nitrate concentration (especially Swiss chard species with 2778.6 ± 1474.7 mg kg^{-1} nitrate concentration) than inflorescence or fruity products. Spinach showed high nitrate concentration after Swiss chard. Samples including spinach, sea beet, and Swiss chard showed nitrite content above the limit of detection.	[21]
Leafy vegetables including savory, parsley, dill, basil, tarragon, leek, and spinach; cucurbit vegetables including turnip, potato, carrot, and onion; fruity vegetables including eggplant, green bean, green pepper, cucumber, tomato, and pepper	Nitrate and nitrite concentration in leafy vegetables was more than fruits and cucurbits. Mint had higher nitrate concentration (74.8 mg/kg) than other leafy vegetables. Average concentration of nitrite in cucurbits was more than fruit vegetables.	[26]
323 samples including stew (102 samples), beverages (116 samples), fruits (55 samples), and vegetables (50 samples)	Nitrate and nitrite concentration was high in celery, while lower levels of nitrate and nitrite were seen in traditionally produced vinegar, verjuice, and tomato.	[22]

Compared to the Iranian regulation (ISIRI no. 16596), a lot of potato, green vegetables, melon, and carrot samples were rejected (20-48%), followed by wheat, lettuce, watermelon, and rice (4-10% rejection). Total nitrite concentration was within the acceptable range in onion and tomato. It might be due to the high permitted level determined by the Iranian regulation. Although, nitrate concentrations were lower than the maximum permitted level (2500 mg/kg) determined by FAO/WHO [22]. What is important is that inappropriate transportation and storage affect nitrite concertation [20], through which nitrate may be converted to nitrite. In this regard, lower concentration of nitrite in uninjured and appropriately stored vegetables has been reported [14]. Therefore, storage condition and climate changes other than the farmlands and fertilizers should be monitored strictly to avoid nitrite/ nitrate accumulation in the agricultural crops in accordance to national and international regulations.

4. Conclusion

High variation in concentration of total nitrite was observed among the agricultural crops studied in the current work. Lettuce and green vegetables were highly contaminated by nitrate/ nitrite, while onion and tomato samples were acceptable according to the Iranian regulation. The other crops were moderately contaminated by the pollutants. Based on our results, strict control and monitoring by the authorities from farm to fork is recommended.

5. Conflict of interest

The authors declare that there is not conflict of interest.

6. Acknowledgment

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References

1. Taghipour H, Hemmati S, Faramarzi E, Somi MH, Dastgiri S, Nowrouze P. Determination of nitrate concentration in consumed vegetables and estimation of that's dietary intake in Shabestar and Khameneh City, northwest of Iran: Azar Cohort study. Progress in Nutrition. 2019; 21: 336-340. https://doi.org/10.23751/pn.v21i1-S.6240

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

3. Bedale W, Sindelar JJ, Milkowski AL. Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. Meat Science. 2016; 120: 85-92. https://doi.org/10.1016/j.meatsci.2016.03.009

4. Santamaria P. Nitrate in vegetables: toxicity, content, intake and EC regulation. Journal of the Science of Food and Agriculture. 2006 ;86(1): 10-17. https://doi.org/10.1002/jsfa.2351

5. Keszler A, Piknova B, Schechter AN, Hogg N. The reaction between nitrite and oxyhemoglobin: a mechanistic study. Journal of Biological Chemistry. 2008; 283(15): 9615-9622. https://doi.org/10.1074/jbc.M705630200

6. Doyle MP, Pickering RA, Dykstra RL, Nelson CL, Boyer RF. Involvement of peroxide and superoxide in the oxidation of hemoglobin by nitrite. Biochemical and Biophysical Research Communications. 1982; 105(1): 127-132. https://doi.org/10.1016/S0006-291X(82)80020-X

7. Kosaka H, Imaizumi K, Tyuma I. Mechanism of autocatalytic oxidation of oxyhemoglobin by nitrite an intermediate detected by electron spin resonance. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology. 1982; 702(2): 237-241. https://doi.org/10.1016/0167-4838(82)90508-8

8. Tamme T, Reinik M, Roasto M. Nitrates and nitrites in vegetables: occurrence and health risks. Bioactive Foods in Promoting Health. Elsevier. 2010; 307-321.

9. Mirvish SS. Blocking the formation of N-nitroso compounds with ascorbic acid *in vitro* and *in vivo*. Annals of the New York Academy of Sciences. 1975; 258(1): 175-180. https://doi.org/10.1111/j.1749-6632.1975.tb29277.x

10. Iranian National Standard Organization. Fruit, vegetable and derived products- Determination of nitrite and nitrate content– Molecular absorption spectrometric method. ISIRI no. 16596. 2013.

11. International Standard Organization. Fruit, vegetable and derived products– Determination of nitrite and nitrate– Molecular absorption spectrometric. ISO no. 6635. 2000.

12. Alonso A, Etxaniz B, Martinez M. The determination of nitrate in cured meat products. A comparison of the HPLC UV/VIS and Cd/spectrophotometric methods. Food Additives & Contaminants. 1992; 9(2): 111-117. https://doi.org/10.1080/02652039209374054

13. Maynard D, Barker A, Minotti P, Peck N. Nitrate accumulation in vegetables. Advances in Agronomy. 1976; 28: 71-118. https://doi.org/10.1016/S0065-2113(08)60553-2

14. Ranasinghe R, Marapana R. Nitrate and nitrite content of vegetables: A review. Journal of Pharmacognosy and Phytochemistry. 2018; 7(4): 322-328.

15. Ziarati P, Arbabi-Bidgoli S. 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. 2014; 4(2): 46-52.

16. Nabrzyski M, Gajewska R. The content of nitrates and nitrites in fruits, vegetables and other foodstuffs. Roczniki Panstwowego Zakladu Higieny. 1994; 45(3): 167-180.

17. Correia M, Barroso A, Barroso MF, Soares D, Oliveira MBPP, Delerue-Matos C. Contribution of different vegetable types to exogenous nitrate and nitrite exposure. Food Chemistry. 2010; 120(4): 960-966.
https://doi.org/10.1016/j.foodchem.2009.11.030

18. Susin J, Kmecl V, Gregorcic A. A survey of nitrate and nitrite content of fruit and vegetables grown in Slovenia during 1996–2002. Food Additives and Contaminants. 2006; 23(4): 385-390.
https://doi.org/10.1080/02652030600573715

19. Chung S, Kim J, Kim M, Hong M, Lee J, Kim C, et al. Survey of nitrate and nitrite contents of vegetables grown in Korea. Food Additives & Contaminants. 2003; 20(7): 621-628.
https://doi.org/10.1080/026203031000124146

20. Petersen A, Stoltze S. Nitrate and nitrite in vegetables on the Danish market: content and intake. Food Additives & Contaminants. 1999; 16(7): 291-299.
https://doi.org/10.1080/026520399283957

21. Matallana Gonzalez M, Martinez-Tome M, Torija Isasa M. Nitrate and nitrite content in organically cultivated vegetables. Food Additives and Contaminants: Part B. 2010; 3(1): 19-29.
https://doi.org/10.1080/19440040903586299

22. Rezaei M, Fani A, Moini AL, Mirzajani P, Malekirad AA, Rafiei M. Determining nitrate and nitrite content in beverages, fruits, vegetables, and stews marketed in Arak, Iran. International Scholarly Research Notices. 2014; 2014.

23. Pirsaheb M, Rahimian S, Pasdar Y. Nitrite and nitrate content of fruits and vegetables in Kermanshah (2010). 2012; 16(1): 76-83.

24. Ding Z, Johanningsmeier SD, Price R, Reynolds R, Truong VD, Payton SC, et al. Evaluation of nitrate and nitrite contents in pickled fruit and vegetable products. Food Control. 2018; 90: 304-311.
https://doi.org/10.1016/j.foodcont.2018.03.005

25. Zhong W, Hu C, Wang M. Nitrate and nitrite in vegetables from north China: content and intake. Food Additives & Contaminants. 2002; 19(12): 1125-1129.
https://doi.org/10.1080/0265203021000014806

26. Afali S, Elahi R. Measuring nitratNie and nitrite concentrations in vegetables, fruits in Shiraz. Journal of Applied Sciences and Environmental Management. 2014; 18(3): 451-457.