original article

# Nitrate and nitrite in leek and spinach from Urmia district and their changes as affected by boiling

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## ABSTRACT

**Aims:** This study was carried out to determine nitrite and nitrate levels in fresh leek and spinach from different greengrocers' shops of Urmia (Iran) and then the effect of boiling and the effect of aqueous boiling pH were studied.

**Materials and Methods:** Nitrite and nitrate content of 15 market samples of leek and spinach from Urmia region were determined by spectrophotometric method. Effect of boiling and their pH levels at home processing condition were studied.

**Results:** Results showed that the fresh vegetables had only traces of nitrite and the level of nitrate was 36-328 ppm $KNO_3$. In the most of samples, nitrite and nitrate contents in spinach were greater than in leek, but lower than standard International Organization for Standardization levels in Iran. Boiling process was carried out, according to home conditions and it caused a decrease in nitrate levels between 23% and 61% in leak and spinach samples, respectively. *T*-test analysis of the boiled vegetables showed a significant reduction about 75% in nitrate content (in dry weight vegetable content), in the samples, but an increase in nitrate content in the boiled water of the sample was observed. The effect pH of boiling (4-8) shows that with an increase in pH, there was a decrease in nitrate contents of boiled water.

**Conclusion:** The experiment showed that the leek and spinach marketed in Urmia region were safe for consumption and boiling of vegetables caused the release of nitrates from vegetables to water after the cooking process. It is of particular importance not to use the vegetable cooking water for use in pureeing homemade baby foods.

**Key words:** Boiled water, boiling, leek, nitrate

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## INTRODUCTION

Nitrate and nitrite contents are an important quality characteristic of vegetables. With respect to its utilization, the spinach and leek are classed in the group of leafy vegetables and potentially disposed to accumulate excessive amounts of nitrates and nitrates.[1,2] Vegetable nitrate and nitrite contents are of interest to governments and regulators



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owing to the possible implications for health.[3,4] Nitrate is a naturally occurring form of nitrogen and is an integral part of the nitrogen cycle in the environment. Nitrate is formed from fertilizers, decaying plants, manure and other organic residues.[5,6] Nitrates are present naturally in soils, water and plants as a consequence of nitrogen fixation. Many studies show that in which all vegetables contain nitrates at varying levels ranging from 1 to 10,000 mg/kg. The wide use of nitrogen based fertilizers in agriculture contributes to the total nitrate present in the environment as well and is produced naturally within the human body.[1] It is also used as a food additive, mainly as a preservative and antimicrobial agent.[7-9] Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture, vegetables and drinking water may contain higher concentrations of nitrate than in the past. The acceptable daily intakes of nitrites and nitrates recommended by World Health Organization is 0-0.06 mg $NO_2$/kg body weight that is equal to 0-3.7 mg $NO_3$/kg body weight.[8] As a result of recent investigations, Walker shows that 75-80% of the daily intake of nitrates derives from vegetables.[9] The only chronic toxic effects of nitrate are those resulting from the nitrite formed by its reduction by bacterial enzymes.[10] It has been estimated that about 4-8% of then nitrate from the diet may be reduced to nitrite by the microflora in the oral cavity.[7] Some studies showed that nitrate exposure is correlated with gastric cancer risk due to the endogenous formation of N-nitroso compounds. The three main sources of nitrate intakes are vegetables, water and cured meat. Vegetables constitute the major dietary source of nitrate, generally providing from 300 to 940 mg/g of the daily dietary intake. Their contribution to nitrite intake is low and in fact lower than that from cured meat products.[5]

Mitigation of nitrate intake may result from processing e.g., washing, peeling and/or boiling.[11,12] According to the International Organization for Standardization in Iran, maximum level of nitrite and nitrate is 4500 mg/k. In China, a suggested maximum level of nitrate in vegetables of 3100 mg/kg has been established.[13]

High intake of nitrite and nitrate may threat human health. Some studies in Iran were conducted to measurement of nitrite and nitrate in Iran, but there are limited studies on nitrite and nitrate content of fruits and vegetables in west Azerbaijan (Urmia) and we need to conduct more research on daily-consumed foods so this survey is important for Iranian Government.

Cooking vegetables tends to lower nitrate content since nitrate is soluble and readily leaches into cooking liquids. Researchers have found that between 14% and 79% of the nitrate contained in fresh vegetables is lost when they are cooked.[12]

The objective of this study was to determine of nitrite and nitrate levels in leek and spinach in 2011-2012 in Urmia markets and the effect of boiling and pH of boiling on both type of product and the effect of boiling of vegetable on nitrate of vegetable cooking water was studied.

## MATERIALS AND METHODS

### Sample collection

A total of 15 market samples of leek and spinach leaves weighting 500 g, taken from a different area in Urmia in Iran, were analyzed for their nitrite and nitrate contents. The samples for nitrate and nitrite content determinations were taken from 20 g of these average samples. Fresh vegetables were analyzed on the day of purchase. All chemicals were analytical grade and the water was de-ionized prior to distillation by application of anionic and cationic resins.

### Nitrite determination

A total of 20 g of each sample leaves was measured and mixed with 80 ml of double-distilled water. About 10 mg of mercuric chloride was added as deproteinizer and allowed to stand for 15 min. the mixture was shaken vigorously for 5 min until fine slurry was formed, the slurry was then filtered through Whatman No.32 filter paper and a clear solution of the sample extract obtained. The nitrite content was determined by a spectrophotometric method developed by Hunt for measuring nitrite in fresh vegetables, which makes possible an accurate determination of nitrite due to simultaneous suppression of interferences from ascorbic acid, tannins (as tannic acid) and nitrate reductase.[13] The base of the method is the diazotization/coupling reaction between sulfanilamide and N (1 naphthyl) ethylenediamine. The absorbance of the colored product was measured at 540 nm with ultraviolet-visible spectrophotometer (Perkin Elmer, Lambda 20). Calculation of nitrite concentration was carried out using a calibration graph.

### Nitrate determination

Nitrates were determined in the same supernatant solution by the ion-chromatography method using Dionex DX-100 ion-chromatograph equipped with a conductivity detector. The conditions of determination were following: Separation was performed by using an exchange column AS9-SC equipped with protection column AG9-SC, the pressure in the system was 14.5-16 Mpa and sensitivity of the instrument was 30. The fluent was 1.8 mM $Na_2CO_3$/1.7 mM $NaHCO_3$ with a flow rate 2.0 ml/min. The 50 mM of $H_2SO_4$ was used to regenerate the anion self-regenerating suppressor (ASRS) self-reacting suppresser. The detection limit of nitrate determination at a signal-to-noise ratio of 3 was 0.01 lg $NO^{-3}$/ml of the final solution or 26 lg $NO^{-3}$/kg FW (Fresh Weight) that is 36 lg $NaNO^3$/kg FW. Recovery was 98.6% and CV 2.84%.[14]

The result is calculated from the calibration curves constructed using the standard solutions. Plot the change in absorbance obtained for the sodium nitrite and potassium nitrate standard solutions on the y-axis against the corresponding nitrite or nitrate concentrations in mg/l on the x-axis. Determine the concentrations of nitrite and nitrate in the sample from the calibration curves using the change in absorbance measured. When the sample has been diluted during preparation, the result multiplied by the dilution factor F. When analyzing solid or semisolid samples that have to be weighed out was calculated the result with respect to the mass of the sample was calculated. In this study, to calculate the content of nitrite and nitrate the equation of 1, 2 were used:

$$\text{Content}_{\text{nitrite}} = \frac{C_{\text{nitrite}} \times 1000}{\text{Mass}_{\text{sample}} \text{ in g/l sample solution}} = \text{mg nitrite/kg} \quad (1)$$

$$\text{Content}_{\text{nitrate}} = \frac{C_{\text{nitrate}} \times 1000}{\text{Mass}_{\text{sample}} \text{ in g/l sample solution}} = \text{mg nitrate/kg} \quad (2)$$

In these equations: The results are determined as sodium nitrite and potassium nitrate.

The conversion factor from $NaNO_2$ to nitrite ($NO_2^-$) is 0.667 and from $KNO_3$ to nitrate ($NO_3^-$) is 0.613.

### Boiling of vegetable

Fresh washed and chopped leaves were cooked in boiling distilled water in stainless-steel pots (1:1, w/v) for 10-15 min. The water was discarded and the leaves cooled prior to chemical analysis. The pH of boiling was set by $H_2SO_4$ and NaOH from 4 to 8 and was controlled during the boiling process by potentiometric methods.

## RESULTS

The amount of dry matter content of each vegetable samples were between 5% and 11%. The nitrate and nitrite amounts determined in leek and spinach in green groceries and the effect of boiling in nitrate and nitrite contents are shown in Table 1, each value being a mean of three parallel determinations and samples. The data in Table 1 shows that nitrite content of leek and spinach products had only traces of nitrites and spinach samples had higher nitrite content than leek. As expected, there were significant differences between leek and spinach nitrite content. In none of the samples, nitrite content was over 10.2 ppm.

The maximum nitrate content was detected in leek and spinach was 328 ppm that is less than Iranian International Organization for Standardization standards. According to the results, concentration of nitrate in spinach was more than leek. The statistical analysis of data of leek and spinach nitrate and nitrite contents of Urmia markets is shown in Table 2.

Boiling of the spinach and leek vegetables caused about 61% and 23% decrease of the total amount of nitrate respectively

**Table 1: Nitrate and nitrite levels in fresh leek and spinach and the effect of boiling**

| Sample no | Fresh | | | | Boiled (dried samples) | | | |
|---|---|---|---|---|---|---|---|---|
| | Leek | | Spinach | | leek | | Spinach | |
| | Nitrite* | Nitrate* | Nitrite* | Nitrate* | Nitrite* | Nitrate* | Nitrite* | Nitrate* |
| 1 | 4.32 | 43.1 | 8.56 | 150 | 2.78 | 29.2 | 7.2 | 88.1 |
| 2 | 5.12 | 56.4 | 7.34 | 210 | 3.28 | 21.8 | 8.1 | 79.5 |
| 3 | 4.35 | 70.6 | 9.2 | 168 | 3.15 | 41.0 | 6.9 | 82.1 |
| 4 | 3.78 | 36.5 | 7.89 | 328 | 2.12 | 39.1 | 7.1 | 69.7 |
| 5 | 3.88 | 38.7 | 6.39 | 215 | 1.58 | 32.8 | 8.3 | 70.2 |
| 6 | 4.15 | 46.1 | 8.01 | 149 | 3.08 | 39.2 | 6.8 | 80.2 |
| 7 | 5.02 | 41.5 | 9.01 | 315 | 2.00 | 42.1 | 8.2 | 81.4 |
| 8 | 3.98 | 38.3 | 7.32 | 204 | 1.50 | 32.5 | 6.4 | 79.6 |
| 9 | 1.85 | 42.1 | 6.56 | 189 | 1.20 | 29.4 | 6.2 | 90.1 |
| 10 | 5.32 | 41.4 | 7.14 | 175 | 2.89 | 21.8 | 7.9 | 86.9 |
| 11 | 4.12 | 39.2 | 8.25 | 132.5 | 1.89 | 19.8 | 8.2 | 51.4 |
| 12 | 3.79 | 51.5 | 8.06 | 145 | 2.08 | 33.1 | 7.5 | 49.8 |
| 13 | 8.12 | 77.4 | 10.2 | 321 | 3.56 | 43.1 | 8.9 | 88.3 |
| 14 | 5.03 | 39.6 | 8.00 | 208 | 3.12 | 22.0 | 7.1 | 79.5 |
| 15 | 6.01 | 42.2 | 7.29 | 214 | 3.09 | 21.4 | 7.2 | 77.0 |
| Mean ± SD | 4.32 ± 0.8 | 42.1 ± 1.8 | 8 ± 1.2 | 204 ± 5.7 | 2.78 ± 0.2 | 32.5 ± 0.8 | 7.2 ± 0.4 | 79.6 ± 2.8 |

*ppm (part per million) or mg/kg; N = 15; significantly different at the level: *P* value < 0.05

**Table 2: Statistical analysis of data on nitrate and nitrite content determined in imported leak and spinach samples**

| Sample | Parameter | Total No. | Mean ± SD mg/kg | Standard deviation | Variation coefficient % | Minimum value mg/kg | Maximum value mg/kg |
|---|---|---|---|---|---|---|---|
| leak | $NO^-_3$ | 15 | 42.1 ± 1.8 | ± 1.801 | 0.2132 | 36.5 | 77.4 |
| | $NO^-_2$ | 15 | 4.32 ± 0.8 | ± 0.8002 | 0.0120 | 1.85 | 8.12 |
| Spinach | $NO^-_3$ | 15 | 204 ± 5.7 | ± 5.712 | 0.51420 | 19.8 | 328 |
| | $NO^-_2$ | 15 | 8 ± 1.2 | ± 1.202 | 0.0132 | 1.20 | 10.2 |

N = 15; significantly different at the level: *P* value < 0.05

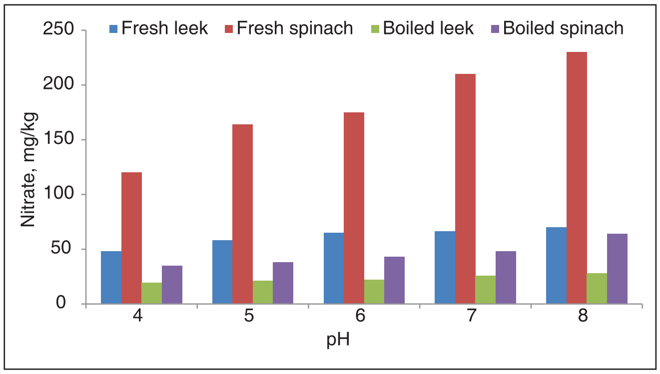


**Figure 1:** The effect of boiling pH in content of nitrate as $KNO_3$ (mg/kg) in vegetable (leek and spinach)

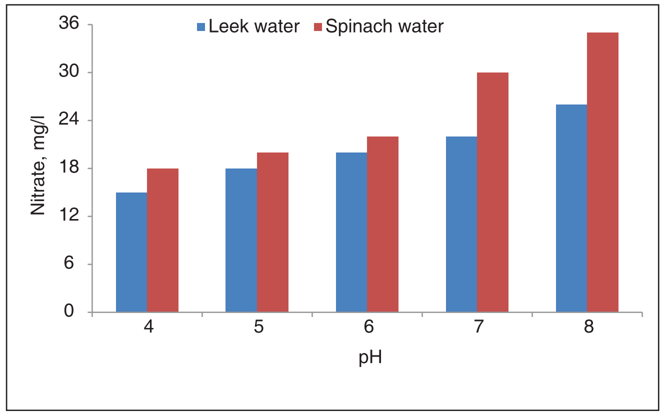


**Figure 2:** The effect of boiling pH in content of nitrite as $KNO_3$ (mg/l) in boiled water

and in corresponding about 29.9% and 4% decrease of the total amount of nitrite in spinach and leek respectively. *T*-test results show that boiling process in 75% of samples have a significant ($P$ value $< 0.0001$) effect in reduction of nitrate, but cause increase in nitrate contents of water boiled. The results of this study show that boiling of vegetables that contain the amount of nitrate is a good process to reduced nitrate content, but caused increased in water boiled. In most of the samples, boiling process cause decrease in nitrite contents. The effect of boiling pH (4-8) shows that with the increase of pH, increase in nitrate content of water and decrease in samples. The effect of boiling pH in content of $KNO_3$ (ppm) in spinach and leek was studied, results show that with the increase of pH releasing of nitrate from vegetable to water was increased and caused the decrease in nitrate in dried vegetable these results are shown in Figures 1 and 2. These results may be because that plant tissues have maximum firmness at pH 4 and decreased firmness at pH values both higher and lower than pH 4.[10]

## DISCUSSION

The experiment showed that the leek and spinach marketed in Urmia region were safe for consumption and boiling of vegetables caused the release of nitrates from vegetables to water after the cooking process. Teresa *et al*. observed that in relation to blanching, the cooking contributed to statistically significant decrease in the contents of nitrates and oxalates.[10] Amal observed an increase in the content of these compounds in blanched or cooked leafy vegetables.[12] Grazyna observed that the losses of total oxalates reached 17-27% after blanching and 24-41% after cooking, always being higher in the case of New Zealand spinach. After 2-min of cooking in tap water.[14] Cooking vegetables cause change in texture of these from hard to soft and lead to release nitrate and nitrite to boiled vegetable water.[13] Boiling process cussed decreased in nitrite and nitrate contents of samples, but decreasing rate in nitrate was more than nitrite and in spinach was more than leek. It was found that between 23% and 61% of the nitrate contained in fresh vegetables was lost when they were boiled. These results indicate that the use of vegetable boiling water for stews, soups, noodle soup, vegetable fricassee (Iranian common food) and gravies might be regarded with some caution, especially for babies and young children, achlorhydria cases and other possible susceptible persons.

**Table 3: The effect of boiling on realizing of nitrite and nitrate from vegetable to water**

| Sample (mg/l) | Distilled water | Water after boiling of leek samples | Water after boiling of spinach samples |
|---|---|---|---|
| Nitrite | 0 | 1.2±0.1 | 2.3±0.4 |
| Nitrate | 0 | 25.4±2.1 | 32.7±4.5 |

Boiling process cussed increasing in nitrite and nitrate of water that used for boiling process these results are shown in Table 3.

According to these results, water that used for boiling have not nitrite and nitrate (deionized water), but after boiling process nitrite and nitrate level in water significantly was increased.

Because, cooking may involve the use of solutions with varying degrees of acidity and/or alkalinity, the effects of solution pH on the nitrogen components of vegetable foods must be understood.

According to the Figures 1 and 2, the effect of boiling pH (4-8) shows that with the increase of pH, increase in nitrate content of water and decrease in samples. The above results confirm the opinion of Grazyna that the losses of nitrates during cooking in water are as much as the functions of the time of cooking, pH and the type of vegetable.[14]

The highest level of nitrate was determined in spinach (328 mg/kg), the lowest in green leek (36.5 mg/kg) and the corresponding levels of nitrite were found in spinach (mean 8 mg/kg) and green leek (mean 4.32 mg/kg) and so in some of the sample. According to the maximum levels that were specified by European Commission Regulation, It showed that the investigated vegetables were safe for consumption. According to Teresa *et al.* and along with the present research results, boiling of Brussels sprouts (starting with boiling water) causes nitrite increase by 84%.[10] Similar effect of the traditional boiling of broccoli on the content of the discussed compounds (only 9% increase) was reported.[10] Boiling potato contributed to 0-60% nitrite losses.[12] Spinach after boiling contained 0-16% less nitrite than the blanched product.[15] This was similar to findings by Shimada and Ko.[16] Since there is a trend now-a-days toward consumption of fresh produce and in particular leafy vegetable varieties, a conservative approach was adopted. Thus, the potential decreases in nitrate concentrations due to processing were not considered for the initial exposure calculations, but can be considered as mitigating factors in a range of mixed vegetable consumption scenarios.

## CONCLUSION

Nitrite and nitrate contents in spinach were greater than in leek, but lower than standard International Organization for Standardization levels in Iran. Boiling process tended to lower nitrate content since nitrate is soluble and readily leaches into water. So, cooking of vegetables has not significant effect on nitrate and nitrite of vegetable foods. With an increase in pH, there was a decrease in nitrate contents of boiled water. It is of particular importance not to use the vegetable cooking water for use in pureeing homemade baby foods.

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