

## The Measurement of Nitrate and Nitrite Content in Leek and Spinach Sampled from Central Cities of Mazandaran State of Iran

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**Abstract:** Vegetables especially leafy ones are the major source of nitrate and nitrite in human diet. Because of the potential health hazards result in high intake of nitrate and nitrite, determination of these ions content in vegetables the concentration has been considered and measured in many countries. The aim of this research was to determine the concentration of nitrate and nitrite in leek and spinach sampled from three central cities of Mazandaran state (IRAN). So, 12 samples of each vegetable have been gathered from farmlands of 4 geographical regions in each city. At last, we assessed 36 leek samples and 36 spinach samples. The measurement was based on ISO Method (NO.6635). Data has been analyzed with T-test and ANOVA. According to the results, the average of nitrate and nitrite content in all of the samples was less than standard limits. Qaemshahr-sampled leek had significantly higher nitrate content than 2 other cities ( $p < 0.01$ ), on the other hand, spinach sampled from Sari had significantly lower nitrate content than 2 other cities ( $p < 0.05$ ).

**Key words:** Mazandaran · Nitrite · Nitrate · Vegetables

### INTRODUCTION

In recent years, an increasing interest in the determination of nitrate levels in food products has been observed, essentially due to the potential reduction of nitrate to nitrite, which is known to cause adverse effects on human and animal health. In fact, nitrite in may react with secondary amines to form toxic and carcinogenic nitrosamine compounds. In addition, nitrite is also known to cause methemoglobinemia (oxygen deficiency) in infants [1, 2, 3]. Fresh and processed vegetables, specially leafy ones, have often been cited as the major sources of dietary nitrate intake, owing to their nitrate accumulation capacity [4, 5, 6]. Nitrite content in vegetables is usually very low as compared to nitrate [7]. Then, in the past few years, efforts have been made to study nitrate accumulation in vegetables and the factors that might influence its occurrence. Actually, the amount of available nitrate in soil (which may be related to the amount of commercial fertilizers applied) appears to be a major factor determining the nitrate concentration level in vegetables [5, 8]. In 1997, to eliminate trade barriers across the European Union, European Commission Regulation (EC)

No. 194/97 set harmonized maximum levels for nitrate in some vegetables. The limits vary according to season, with higher nitrate levels permitted in winter-grown vegetables [9, 10].

At the end, the objectives of this project were to 1) determine the amount and the variability of the nitrate and nitrite content in leek and spinach grown in farmlands of Sari, Qaemshahr and Babol cities by UV-Visible Spectrometry, 2) evaluate the relative safety of these leafy vegetables based on the maximum levels of European Commission Regulation (EC) No. 194/97.

### MATERIALS AND METHODS

**Sampling:** Vegetables were sampled from local farmlands of Sari, Qaemshahr and Babol between Sep.23 to Nov.23, 2006. Four geographical areas were chosen in each of cities (north, south, east and west) and in each area, three samples of each vegetable were gathered randomly. Samples were carried to laboratory in iceboxes and were analyzed as soon as possible. If storage was necessary, samples were stored at  $-4^{\circ}\text{C}$ .

**Apparatus:**

- Spectrophotometer UV/Visible, model: Ultrospec 4000 for use at 538 nm, providing a light path of 1 cm or longer.
- Reduction column purchased from Kimble Kontes Co. (USA), filled by Cadmium coarse powder 0.3-0.8 mm (Merck). Preparation and efficiency test of cadmium column was based on ISO Method (No.6635) [11].

**Reagent and solution:** All chemicals used, were of analytical reagent grade purchased from Merck Co. (Germany). Deionized water (with a specific conductance of less than 0.1  $\mu\text{S cm}^{-1}$ ) was used throughout.

**Procedure:** The measurement method was based on ISO Method (NO.6635) [11]. 10 g from 200 homogenized vegetables weighed precisely, added hot deionized water (80°C) and 5 ml saturated tetra borate and heated on water bath for 15 min. After that the aqueous extract was clarified with Potassium hexacyanoferrate and Zinc acetate, slurry transferred to 200 ml volumetric flask and diluted by deionized water, then filtrated.

**Determination of nitrite content:** 10 ml aliquot of filtrate was transferred to a 50 ml flask. After developing of purple color due to addition of sulfanilamide and *N*-(1-naphthyl)ethylene diamine dihydro chloride, diluted to volume 50 ml and measured the absorption in 538 nm on the standard curve with linear equation:  $y = 0.015 + 0.022x$ , ( $r^2=0.999$ ).

**Determination of nitrate content:** A second 10 ml aliquot of filtrate was mixed with buffer (pH=9.6) and passed through the reduction column. Column was washed with ca. 15 ml deionized water. The eluate and wash were collected in a 50 ml volumetric flask and continued the rest as like as nitrite procedure.

**Statistical Analysis:** The nitrate and nitrite content in two vegetables of three cities analyzed descriptively and  $p < 0.05$  set as significant differences to one-way ANOVA and T-test of means. All statistical analyses were done by SPSS software for windows Ver.14.

**RESULTS**

The average of nitrate content in spinach and leek which sampled from four different geographical areas in each city was investigated and compared together (Table 1).

Table 1: Summary of nitrate content in spinach and leek ( $\text{NO}_3$  mg  $\text{kg}^{-1}$  FW) in four geographical areas of three cities ( $p < 0.01$ ).

City	Region	N	Spinach	Leek
			Mean $\pm$ S.D.	Mean $\pm$ S.D.
Babol	North	3	401 $\pm$ 60	0.00
	West	3	351 $\pm$ 10	0.00
	South	3	310 $\pm$ 61	0.00
	East	3	188 $\pm$ 9	0.00
Qaemshahr	North	3	457 $\pm$ 44	86 $\pm$ 14
	West	3	364 $\pm$ 28	60 $\pm$ 3
	South	3	194 $\pm$ 23	83 $\pm$ 4
	East	3	439 $\pm$ 37	79 $\pm$ 22
Sari	North	3	24 $\pm$ 5	0.00
	West	3	150 $\pm$ 3	0.00
	South	3	346 $\pm$ 36	0.00
	East	3	282 $\pm$ 27	0.00

Table 2: The total average of nitrate content in spinach and leek ( $\text{NO}_3$  mg  $\text{kg}^{-1}$  FW) of 3 cities ( $p < 0.05$ )

City	N	Spinach*	Leek**
		Mean $\pm$ S.D.	Mean $\pm$ S.D.
Babol	12	313 $\pm$ 89	0.00
Qaemshahr	12	364 $\pm$ 112	77 $\pm$ 16
Sari	12	223 $\pm$ 129	0.00

\*  $p < 0.02$ , \*\*  $p < 0.01$

According to the nitrate content in spinach, there was a significant difference between east of Babol, south of Qaemshahr, north of Sari and the other 3 regions of each city ( $p < 0.01$ ). But no significant difference was found in the average of nitrate content between leek samples of Qaemshahr.

Total average of nitrate content in spinach was 312, 363 and 223 ( $\text{NO}_3$  mg  $\text{kg}^{-1}$  FW), in Babol, Qaemshahr and Sari, respectively ( $p < 0.01$ ). On the other hand, leek samples contained 0.0, 76, 0.0 mg/kg FW of nitrate, in Babol, Qaemshahr and Sari, respectively (Table 2). Qaemshahr-sampled leek had significantly higher nitrate content than 2 other cities ( $p < 0.01$ ) and spinach sampled from Sari had significantly lower nitrate content than 2 other cities ( $p < 0.05$ ).

The amount of nitrite in all samples of both vegetables (spinach and leek) in all 3 cities was very low and not detectable.

**DISCUSSION**

The tissue nitrate levels for spinach and leek recorded in this study are comparable to the levels reported elsewhere [12-14]. A research was done in

Esfahan-IRAN (Fall 1998- Summer 1999) showed that the mean of nitrate in some leafy vegetables was 287.9 ppm [15]. The European Union set maximum limit for nitrate and nitrite in fresh spinach (harvesting during 1 November to 31 March) is 3000 and 0.0 ppm, respectively [9]. In addition, this limit for nitrate and nitrite in leek is 2500 and 0.0 ppm respectively. Corresponding to the results (Table 1 and 2) the average of nitrate and nitrite concentration in all spinach and leek samples of three cities was less than the maximum levels that specified by European Commission Regulation. This means that the investigated vegetables are safe for consumption. According to Table 1, the mean of nitrate content in spinach samples from north of Babol, north and east of Qaemshahr, east of Sari were more than other areas in each city. Also Qaemshahr-sampled spinach and leek had more nitrate content than other two cities. These results can be explained as follows:

- Vegetable nitrate levels are affected by the rate and type of nitrogen fertilizers applied and by soil nitrification activity, soil texture and harvest time [16].
- Water that use in this areas is from flowing water unlike the other areas that use well water for irrigation.
- Much rain and over flowing water of other areas to the mentioned areas, cause to accumulation of nitrate [17].

#### **Suggestion:**

- Measurement of nitrate and nitrite in agricultural products should be regularly performed according to per capita consumption of fertilizer.
- According to have not any standard level for vegetables nitrate content in IRAN, seems necessary to establish specific maximum standard level considering to different condition in our country.
- Fertilizing and irrigation practices that produce vegetables with low nitrate content compatible with optimum yield must be developed [18, 19].
- Some methods such as preplant soil nitrate testing, compost based fertility management, afternoon to evening harvest, may reduce nitrate concentration in vegetables [20, 21].
- Educate the people, about the better preparation and storage of edible part of vegetables (Like petiole removal).

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