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Nitrate Determination of Vegetables in Varzeghan City, Northwestern Iran

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ABSTRACT

Background: Vegetables play an important role in human nutrition. Nitrate content is a significant quality criterion to determine characteristic of vegetables. About 80% of nitrate intake in human is from vegetables and fruits. High dietary intake of nitrate is seen as an undesirable because of its association with gastric cancer and infantile methemoglobinemia. Varzeghan, Northwestern Iran is one of the cities with high Age-standardized incidence rates (ASR) of gastric cancer in Iran. Currently, in Varzeghan there is no available and accurate information describing nitrate concentration as one of the important risk factors of vegetables for human consumption.
Methods: In this cross sectional study totally 11 types of vegetables (cabbage, lettuce, spinach, parsley, coriander, dill, leek, fenugreek, tarragon, fumitory and mint) from several different greengrocery of Varzeghan were collected in spring (April) and autumn (November and December) 2011 and their nitrate contents were analyzed.
Results: Mean nitrate contents at the above noted fresh vegetables were 161, 781, 83, 707, 441, 501, 1702, 684, 805, 772 and 191 mg $NO_3^-kg^{-1}$ respectively. In none of the 11 fresh vegetables nitrate content were not more than established limitations.
Conclusion: Nitrate concentrations were below of others reported at different countries. The mean concentration of nitrate at all vegetables in autumn was higher than in spring significantly.
Keywords: Nitrate, Vegetables, Nutrition, Iran

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Introduction

Nitrogen is necessary for growth of plants and nitrate is part of the nitrogen cycle compounds [1]. Nitrate contamination in vegetables occurs when crops absorb more than they required for growth. Some vegetable can be stored high nitrate in their organs [2]. Vegetables play an important role in human nutrition since they are an outstanding source of vitamins, minerals and biologically active compounds. Nitrate content is a significant quality criterion for determination of characteristic of vegetables [3, 4]. Although nitrate is apparently

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non-toxic below maximum residue levels (MRLs), it may be endogenously transformed to nitrite that can react with amines and amides to produce N-nitroso compounds [5]. These have been related to an increased risk of gastric cancer [1, 6, 7]. Stomach is most at risk from endogenous N-nitroso compound synthesis. High nitrate intake was associated with gastric cancer in England, Colombia, Chile, Japan, Denmark, Hungary, Italy and in the Van region of Turkey [8, 9]. Nitrates can also have a teratogenic effect and with the formation of methemoglobin the oxygen transmis-sion to tissues is disrupted [4]. Lethal dose of the incoming nitrate from food is 330 mg per kg of body weight [10]. Thus intake of nitrate is seen as an undesirable component because of its association with gastric cancer. Off course some recent researches suggest that dietary nitrate may have beneficial effects; based on the hypothesis that nitric oxide formed in the stomach from dietary nitrate has antimicrobial effects on gut pathogens and a role in host defense [4].

There are several factors affecting NO_3 uptake and accumulation in vegetable tissues, e.g., (day) light intensity, type of soil, temperature, humidity, frequency of plants in the field, plant maturity, genetic, harvesting time, size of the vegetable unit, storage time and source of nitrogen [5,11,12]. Of the factors studied, nitrogen fertilization and light intensity have been identified as the major factors that influence nitrate content in vegetables [4]. Nitrate reductase enzyme activity during the day can reduce nitrate. Therefore it is better to harvest vegetables in the evening [13]. About 80% intake of nitrate in human is from vegetables and fruits [14]. In Ardabil the proportion of vegetables and fruits in nitrate entering the body was about 60% [15]. In many European countries and Australia limits for nitrate in some food has been determined, but In Iran there is no limit for nitrate in vegetables and foods [16].

Varzeghan is one of the cities with high age-standardized incidence rates (ASR) of gastric (stomach) cancer in Northwest of Iran [17]. Currently, in Varzeghan City there is no available and accurate information that describes the nitrate concentration as one of important risk factors in vegetables. Thus in this study nitrate concentration in vegetables collected from city's greengroceries of Varzeghan was determined and compared with the concentration reported in the literature.

Materials and Methods

In this cross sectional study, 22 (11 type vegetable that twice repeated) vegetable samples (cabbage, lettuce, spinach, parsley, coriander, dill, leek, fenugreek, tarragon, fumitory and mint) from several different greengroceries of Varzeghan, were collected in spring (April) and autumn (November and December), 2011. Each sample was collected from at least 2-5 greengroceries then were mixed with each other and considered as one sample. After transmission of the samples to the laboratory, they were washed by tap water and rinsed with distilled water. Then similar vegetables mixed together and after drying at laboratory temperature, were dried in an oven at temperature of 80 °C [18]. Dried vegetables were powdered by grinding and passed from mesh No. 20. Then 0.4 g meshed vegetable with 0.02 g activated carbon added into the flasks and 40 mL of 0.025 M of aluminum sulfate solution added and shacked for 30 minutes. Then the solution was passed through Whatman filter paper 42. Then 0.02 g of activated carbon was added to extracted solution again, and repeated the extraction cycle [18]. After extracting of samples, for determination of nitrate, Cataldo method was used and by spectrophotometer at a wavelength of 410 nm nitrate levels were detected [19-21]. To prevent the changes in nitrate concentrations after the extracts, the extracted solution immediately were measured. To ensure about nitrate measurement in prepared samples, the analyzing were repeated two times in all of the samples and average was considered as concentration of each sample. All reagents used in this study were of analytical grade. De-ionized water was used for preparing all the solutions. Potassium nitrate (Merck), activated carbon

(Merck), aluminum sulphate (Merck), sodium hydroxide (Merck) and acid salicylic (Sigma) were used .

Results

The concentration of nitrate in studied vegetables consumed by Varzeghan citizens in two seasons is presented in Table 1. The average of nitrate concentration of two season indicated that Tarragon with 83 mg kg^{-1} and Fumitory with 1702mg kg^{-1} had the minimum and highest rate of nitrate at vegetables respectively.

Table 1: Nitrate concentration at vegetables (mg NO_3-kg^{-1})

Vegetables	Spring	Autumn	Mean of two seasons
Cabbage	133	189	161
Lettuce	767	794	781
Tarragon	26	139	83
Coriander	240	1173	707
Parsley	297	585	441
Spinach	692	310	501
Fumitory	972	2431	1702
Mint	197	1170	684
Fenugreek	852	757	805
Dill	634	909	772
Leek	79	302	191

Nitrate increased in autumn in most vegetables. Statistical analysis was performed between means of concentration nitrate in spring and autumn in vegetables with the two-sample *t*-test at α=0.05 and determined that nitrate contents in autumn were higher significantly (P<0.05).

The obtained results indicated a considerable variation in the nitrate contents within the same vegetable species (Table 2). Except Fumitory (1702 mg kg^{-1}) nitrate concentration in the other vegetables was less than 1000 mg kg^{-1}. Also nitrate content at vegetables in spring was lower than the autumn.

Discussion

Many studies were conducted to measure nitrate content in vegetables. Cultures are grown and large quantities of fertilizers are used. Vegetables play an important role in the population's diet. The vegetables selected for this study are some of the most commonly consumed and also the ones that were available during the collection period. Individual results obtained for nitrate levels on the studied samples are presented in Table 1. The levels of the nitrate obtained in this study from vegetable were compared with previous studies (Table 2). There is a considerable variation in the nitrate contents within the same vegetable species reported at the previous researches. As previously described, the nitrate content in vegetables depends on many factors. The average nitrate contents for cabbage in current study was notably lower than the results obtained for cabbages in other countries and city of Iran (Table 3), particularly those reported in China and Korea [16, 22, 23].

For lettuce the mean nitrate value obtained was 781 mg kg^{-1} while comparing with the nitrate contents presented in Table 3, these are in the range of those found for lettuce in Brazil and Greece [24, 25] and is closer to the average nitrate content of China and Ardabil of Iran [16, 26]. The European Commission has established maximum levels (MLs) for nitrate in lettuce (2500–3500 mg kg^{-1} for summer harvest and 4000–4500 mg kg^{-1} for winter harvest) [27].

In our study, the lowest nitrate value was obtained for a tarragon sample and highest nitrate for fumitory with 1702 mgkg^{-1}. In literature review we could not find similar study that show tarragon as well as fumitory nitrate content.

The mean nitrate obtained for coriander in this study and in the study conducted in Ardabil of Iran is very low compared to other countries like China and the study of European food safety authority (EFSA) with [2, 22].

Nitrate content of Spinach in this study is closer to sample that studied to Brazil but has a considerable difference with other country studies such as turkey and Greece [24, 25, 28].

Table 2: Comparison of nitrate contents (mg NO -kg^{-1}) in fresh vegetables from this study with other researches

Vegetables	Mean concentration of nitrate reported in the literature		Mean nitrate content in Varzeghan
	country	Nitrate	
Cabbage	Ardabil of Iran [16]	326	161
	Greece [25]	209	
	China [22]	1530	
	Korea [23]	730	
Lettuce	Ardabil of Iran [16]	618	781
	Greece [25]	282	
	Brazil [24]	1420	
	China [26]	896	
Tarragon	--	--	83
Coriander	Ardabil of Iran [16]	873	707
	China [22]	3200	
	European country [2]	2445	
Parsley	Ardabil of Iran [16]	945	441
	Turkey [28]	1204	
	Estonia [32]	966	
	France [33]	1890	
Spinach	Ardabil of Iran [16]	1021	501
	Greece [25]	1250	
	Brazil [24]	528	
	Turkey [28]	2820	
Fumitory	--	--	1702
Mint	Isfahan of Iran [29]	872.4-3386.1	684
Fenugreek	Isfahan of Iran [29]	821.9-3400.3	805
Dill	Ardabil of Iran [16]	747	772
	EFSA [2]	1332	
leek	Ardebil of Iran [16]	324	191
	European country [2]	345	
	Korea, conventional farming [1]	538	
	Korea, organic farming [1]	1450.3	

In addition nitrate concentration is lower than the Isfahan of Iran and maximum level of European commission for spinach is 2500 mg kg^{-1} for summer harvest, and 3000 mg kg^{-1} for winter harvest [27, 29]. In China, a suggested maximum level of nitrate in vegetables of 3100 mg kg^{-1} has been established [4]. It is clear in our study that except fumitory other vegetable have lower than 1000 mg kg^{-1} nitrate content even leek, tarragon and cabbage have lower than 300 mg kg^{-1} nitrate. Vegetables were shown to

contain nitrates at varying levels in different seasons [30]. This was in agreement with the result of this work. Nitrate in vegetables in autumn and spring has changed and significant variation ($P<.05$) between the mean of nitrate in spring and autumn were obtained (Table 2).

Seasonal differences in nitrate concentrations were observed for leek, lettuce, mint, tarragon, fumitory, parsley, cabbage, coriander and dill samples but not for fenugreek or spinach. According to report of Ministry of Agriculture, Fisheries and Food, UK (MAFF) concentration of nitrate in some vegetable varied in different season. For example mean content of nitrate in lettuce, spinach and cabbage in spring are 337, 1382 and 318 mg kg^{-1} and in autumn are 1205, 2259 and 450 mg kg^{-1} respectively [31]. In our study unlike MAFF report, nitrate level in spinach in autumn was higher than in spring. According to Kaminishi et al. concentration of nitrate in spinach in spring was lower than in autumn (4100 and 4300 mg kg^{-1} respectively). Temperature and light intensity are important environmental factor that can influence on the nitrate metabolism of spinach. It is reasonable that distribution pattern of nitrate concentration differ greatly within a cultivation seasons [30].

The apparent variability in current study may be explained by the different sources of the vegetable samples, light intensity and other environmental factors that affect the nitrate concentrations in vegetables.

Conclusions

Increasing concern about nitrate toxicity has produced a number of studies on nitrate concentration of fresh vegetable samples. Nitrate levels of 11 type fresh vegetable from Varzeghan in autumn and spring were determined and their levels compared to those reported in recent literature. In none of 11 fresh vegetable the nitrate content were not more than established limitation. Even the nitrate concentrations were below of others reported in different countries. The mean concentration of nitrate in vegetables in autumn was higher than in spring significantly.

Therefore according to results the amount of nitrate in vegetables in the studied area is lower than other studies, and the high incidence of gastric (stomach) cancer in Varzaghan cannot be due to the nitrate level in vegetables.

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