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Nitrate and Nitrite Contents of Some Vegetables Consumed in South Province of Turkey

[1]Firdevs Mor, [1]Fatma Sahindokuyucu and [2]Neslihan Erdogan
[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine,
University of Mehmet Akif Ersoy, 15100, Burdur, Turkey
[2]Department of Biology, Faculty of Arts and Sciences,
University of Mehmet Akif Ersoy, 15100, Burdur, Turkey

Abstract: A scientific basis for the evaluation of the risk to public health arising from excessive dietary intake of nitrate in south Province of Turkey is provided. The nitrate (NO_3^-) and nitrite (NO_2^-) contents of various vegetables (cabbage, leek, lettuce, parsley, spinach and radish) are reported. Seven four samples of 6 vegetables cultivated during different seasons were analysed for nitrate and nitrite by ultraviolet spectrophotometry. The mean nitrate levels were higher in radish (3428 mg kg^{-1}) and lettuce (1439 mg kg^{-1}), intermediate in spinach (1132 mg kg^{-1}), parsley (1070 mg kg^{-1}) and lower in cabbage (510 mg kg^{-1}) and leek (40 mg kg^{-1}) compared with those in other vegetables. The results of the study show that nitrite contents in vegetables ranged from 0.20-28.80 mg kg^{-1}. It was observed that nitrate contents in vegetables varied depending on the type of vegetables and were lower than that of similar vegetables grown in other countries. From the results of the studies and other information from foreign sources, it can be concluded that it is not necessary to establish limits of nitrates contents of vegetables cultivated in Turkey due to the co-presence of beneficial elements such as ascorbic acid, tocopherols, carotenoids and flavonoids which are known to inhibit the formation of nitrosamine.

Key words: Nitrate, nitrite, vegetables, dietary intake, vegetables, Turkey

INTRODUCTION

Nitrates are present naturally in soils, water, plants (particularly in vegetables) as a consequence of nitrogen fixation. The wide use of nitrogen based fertilizers in agriculture contributes to the total nitrate present in the environment as well. The significance of nitrate to human health derives from the fact that nitrate can be converted *in vivo* to nitrite producing toxic effects.

In addition, nitrite ion can react with secondary or tertiary amines to form N-nitroso compounds, some of them being implicated in the etiology of cancers (Sebecic and Vedrina-Dragojevic, 1999). Nitrate contamination in vegetables occurs when crops absorb more than they require for their sustainable growth. Spinach, lettuce, broccoli, cabbage, celery, radish, beetroot, etc., possess the tendency to accumulate nitrates. On the other hand, vegetables such as carrots, cauliflower, French beans, peas and potatoes seldom accumulate nitrates.

Nitrate content of vegetables may range from 1-10,000 mg kg^{-1} (MAFF, 1998; Ximenes *et al*., 2000). Concentrations of nitrate in vegetables depend on agricultural practices, storage conditions, the temperature and light in which they are grown and the concentrations of nitrate in the soil, fertilizers and water used to grow the vegetables (National Research Council, 1981; Duncan and John, 2006). The main concern for the public health is the link between nitrates and stomach cancer. Nitrite is a precursor in the formation of nitrosamines (Tannenbaum and Correa, 1985). Another important concern is that vegetables are an important part of most babies diets (Huarte-Mendicoa *et al*., 1997). Young babies with low stomach acidity may suffer from infantile methemoglobinemia due to excessive nitrates in their diet where nitrite is substituted for oxygen in hemoglobin and death may occur (Ezeagu, 1996; Gundimeda *et al*., 1993). Even after such a high risk on public health there is no data available on South Province of Turkey's commonly consumed vegetables. This forms the basis for the regular monitoring of nitrate and nitrite levels in commonly consumed South Province of Turkey's fresh vegetables.

MATERIALS AND METHODS

Preparation of samples: Samples were washed with tap water. The edible part of the samples was used for analyses. For each vegetable, a composite sample of many individuals (e.g., spinach, cabbage, leek, lettuce, parsley

Corresponding Author: Firdevs Mor, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, 15100, Burdur, Turkey

and radish) was used. After washing the vegetables were chopped into small sections and homogenized in a blender mill.

Extraction and analysis: Sen and Donaldson (1978) method was used for the extraction of nitrate-N and nitrite-N in the present study. Homogenized sample (10 g) was accurately weighed and blended for 5 min with 70 mL of water. Then 12 mL of 2% NaOH was added while pH ca. About 8 was adjusted with 2% NaOH (avoiding excess NaOH). The slurry was transferred to a 200 mL volumetric flask and heated on water bath (50-60°C) with occasional swirling until the temperature of the suspension reached about 50 C. $ZnSO_4$ (10 mL) was added and temperature of the suspension maintained at about 50°C for further 10 min. If a white precipitate of $Zn(OH)_2$ did not appear, 2-5 mL of 2% NaOH was added (avoiding excess NaOH). Contents were cooled to room temperature by immersing flask in cool water bath. The solution was diluted to a fixed volume with water and mixed thoroughly. Then the solution was filtered through a 0.45 mL membrane filter. The first zone of filtrate was discarded in order to overcome possible nitrate contamination from the filter-papers. The limit of detection was 5 mg kg^{-1} for nitrate and 0.3 mg kg^{-1} for nitrite.

Nitrate analysis: An aliquot of 10 mL filtrate was mixed with 5.0 mL NH4Cl buffer and passed through the cadmium column. This solution was reacted with sulphanilamide and N-(1-naphthyl) ethylene-diamine and the absorbance of the violet azo compound was measured at 540 nm (Sen and Donaldson, 1978).

Nitrite analysis: The nitrite was determined calorimetrically by diazotization of sulfanilamide and subsequent coupling with N-(lnapthyl)-ethylenediamine to form a pink azodye whose absorbance was measured at 540 nm against aqueous prepared standards.

Quality assurance: The method was shown to provide accurate results by participation in the UK Food Analysis Performance Scheme (FAPAS) exercises. The results for the nitrate contents of 1052 mg kg^{-1} spinach puree were Z:-0.7 and robust mean was 1097.3 mg kg^{-1} (FAPAS Secretariat, 1998).

RESULTS AND DISCUSSION

Nitrate contents of vegetables: The nitrate levels of all vegetables measured in this study are shown in Table 1. Generally, nitrate contents of all samples were found very high compared with nitrite values. It was shown that radish; spinach, lettuce and parsley contained a higher level of nitrates whilst cabbage and leek contained a lower level of nitrates. The mean contents of nitrates are summarized as follows (mg kg^{-1}): Cabbage, 510; leek, 91; lettuce, 1439; parsley, 1070; spinach, 1132; radish, 3428. The content of nitrates can vary from 1-10000 mg kg^{-1}, depending not only on genetic factors such as kinds or strains of the vegetables but also on environmental factors including the places or conditions of cultivation and storage (European Commission, 1995; World Health Organization, 1995).

Generally, the levels of accumulated nitrates needed for subsequent survival and growth fluctuate in different vegetables and in the different parts of the vegetables. A higher nitrate level was observed in greenish-yellow leaf vegetables (European Commission, 1995; World Health Organization, 1995). Generally, a lower level of nitrate was observed in the vegetable groups of cucumbers and tomatoes (<500 mg kg^{-1}) while higher levels were observed in the groups of lettuce, spinach, radish and Chinese cabbage (Scharpf, 1991). The highest levels of nitrate were in radish followed by lettuce, spinach, parsley, cabbage and leek.

Nitrite contents of vegetables: As shown in Table 1, the mean nitrite contents in most vegetables were <15 mg kg^{-1}. Nitrite contents were the highest in lettuce and pollution levels decreased in the order of spinach, radish, cabbage, parsley and leek. All samples were detected for nitrite. The highest nitrite concentrations were mainly detected in lettuce from 2.92-8.80 mg kg^{-1} (mean value 11 mg kg^{-1}). It was also detected in some only samples of spinach, radish and cabbage (from 0.06-0.23 for cabbage, from 0.15-0.32 for spinach and from 0.15-0.41 mg kg^{-1} for radish). In the samples of leek and parsley the least nitrite concentrations were detected.

Comparison with monitoring results of foreign countries: The levels of the nitrate and nitrite in vegetables in some other parts of the worlds are shown in Table 2. The results in this table are on the basis of their fresh weight and NO_3, NO_2. Comparison with levels in vegetables in the present study revealed that the levels are generally comparable. The results obtained were compared with those of other countries (Table 2). Leafy vegetables (lettuce, spinach, parsley and radish) appear to contain high levels of nitrate and nitrite. This trend is similar to those reported in Korea (Chung *et al*., 2003) and in other countries; Germany (Scharpf, 1991), England (MAFF, 1996), Italy (Santamaria *et al*., 1999). The average nitrate levels of each vegetable varied depending on the country (European Commission, 1995), the average levels

Table 1: Nitrate and nitrite concentrations in vegetables (fresh weight, mg kg^{-1}, NO_3^-, NO_2^-)

	Nitrate				Nitrite			
Vegetables	n	Range	Mean	SD	n	Range	Mean	SD
Cabbage	11	124-819	510	250	11	0.9-3.80	2.25	0.95
Leek	11	24-68	40	11	11	0.2-1.50	0.66	0.35
Lettuce	12	540-3809	1439	949	12	2.9-8.80	11.00	6.86
Parsley	12	234-1701	1070	612	12	0.2-1.50	0.75	0.42
Spinach	18	819-1905	1132	281	18	3.8-12.7	7.18	2.90
Radish	10	952-9259	3428	3172	10	2.4-14.2	6.39	4.26

[a]SD: Standard Deviation

Table 2: Levels of nitrate and nitrite in vegetables from the Turkey compared with previously published results from other parts of the world (fresh weight, mg kg^{-1}, NO_3^-, NO_2^-)

	Nitrate and nitrite concentrations (mg kg^{-1}, fresh weight)												
	Present study		Korea[a]		England[b]		Germany[c]		Japan[d]		Danish [e]		Italy
Vegetables	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
Cabbage	510	2.255	725	0.4	712	0.8	3100	-	1040	0.7	1000	0.16	400
Leek	91	0.664	-	-	-	-	-	-	-	-	330	0.15	-
Lettuce	1439	11.000	2430	0.6	2330	0.6	750-5500	-	-	-	2440	0.14	1089
Parsley	1070	0.758	-	-	-	-	-	-	-	-	-	-	1150
Spinach	1132	7.189	4259	1.0	2470	3.8	900-5400	-	3560	7.0	1743	11.00	1845
Radish	3428	6.390	1878	0.8	2600	0.3	780-2400	-	1060	0.4	-	-	2067

[a]Chung *et al.* (2003), [b]MAFF (1996), [c]Scharpf (1991), [d]Sumiko and Masako (1993), [e]Petersen and Stoltze (1999) and [f]Santamaria *et al.* (1999)

of nitrate of lettuce and spinach varied widely ranging from 907-4674 ppm and from 390-3383 ppm, respectively. The minimum and maximum levels of nitrate and nitrite in lettuce were lower than the amounts reported European Commission (1995). However, the minimum nitrate levels in spinach were higher than those found in EU while the maximum nitrate values were below the amount found European Commission. The nitrite contents in all vegetables were higher than those detected in Korea (Chung *et al*., 2003) and England (MAFF, 1996). The spinach grown in Danish contain higher levels of nitrite compared with the values (while these spinach values were below the amount found in Danish) while the values for nitrite levels in spinach were similar to that recorded in Japan (Sumiko and Masako, 1993).

However, cabbage, leek and lettuce grown in Japan and Danish contain lower levels of nitrite compared with the values. On the other hand, the nitrate and nitrite concentrations in radish were higher than those detected in Korea (Chung *et al*., 2003), Japan (Sumiko and Masako, 1993) and England (MAFF, 1996). The values of nitrate in some vegetables were lower than the amounts reported in Korea (Chung *et al*., 2003), Germany (Scharpf, 1991), Japan (Sumiko and Masako, 1993), Danish (Petersen and Stoltze, 1999). However, the mean levels of nitrate in cabbage, lettuce and radish were higher than those found Santamaria *et al.* (1999) in similar vegetables. But the nitrate levels in other vegetables (parsley and spinach) were lower compared with these obtained in Italy (Santamaria *et al*., 1999).

It was apparent that nitrate levels of cabbage and spinach cultivated in England (MAFF, 1996) and Japan (Sumiko and Masako, 1993) were higher than those in present study however, the levels in radish grown in these countries were lower compared with these obtained in the study. Also, nitrate contents in cabbage, lettuce and spinach produced in Korea (Chung *et al*., 2003) and Germany (Scharpf, 1991) were shown to be higher than those in the present paper's similar vegetable samples. But also the levels of nitrate in radish grown these counties were lower than the values obtained in the study. Monitoring results from the Danish (Petersen and Stoltze, 1999) showed that nitrate contents were higher in cabbage, leek, lettuce and spinach compared with the present study.

CONCLUSION

With the exception of radish, the levels of nitrates in vegetables grown in the present study were lower than the values obtained in other countries. However, the nitrite levels were higher or even similar than those detected in other countries. Moreover, it is believed that antioxidant agents ubiquitous in vegetables play a beneficial role as a inhibitor of the nitrosamine formation from nitrites. Therefore, nitrates consumed from vegetables are concluded to be harmless to human health. In conclusion, overall evaluation of all the studies performed abroad or domestically leads to the belief that it is necessary to establish limits of nitrate contents of vegetables cultivated in Turkey. This review of nitrate and nitrite contamination in vegetables in Turkey shows that this is a serious problem that will continue to grow as increasing amounts of nitrogenous fertilizers are applied.

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