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Survey of Nitrate and Nitrite Levels of Fresh Vegetables in Turkey

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Abstract: In this study, the nitrate and nitrite contents of a total 258 samples of 7 different vegetables (tomato, carrot, head lettuce, leaf lettuce, iceberg, parsley and spinach) sold in Ankara markets in Turkey were determined during the period of 6 months. These vegetables are supposed to provide the major contribution to the intake of nitrate from the diet. Nitrite was estimated spectrophotometrically and nitrate after reduction to nitrite with cadmium column. The highest content of nitrate was found in parsley (1513.36 mg kg^{-1}) followed by spinach (1456.04 mg kg^{-1}), crisphead lettuce (1042.81 mg kg^{-1}), butterhead lettuce (914.22 mg kg^{-1}), iceberg (623.38 mg kg^{-1}), carrot (190.03 mg kg^{-1}) and tomato (11.06 mg kg^{-1}). The mean nitrite values of spinach, parsley, crisphead lettuce, iceberg, butterhead lettuce, carrot and tomato were determined as 2.31, 1.78, 0.98, 0.92, 0.84, 0.65 and 0.36 mg kg^{-1} in fresh weight, respectively.

Key words: Vegetables, nitrate, nitrite, carrot, tomato, spinach

INTRODUCTION

Nitrates are present naturally in soils, water and plant materials as a consequence of nitrogen fixation. Probably, more than 90% of the nitrogen absorbed by plants is in the form of nitrate. The presence of nitrates is one of the consequences of the mechanism by which plants absorb the nitrogen element from fertilizers or organic materials which are essential to the process of protein synthesis (Chung *et al*., 2003; Meah *et al*., 1994; Santamaria *et al*., 1999).

Nitrate and nitrite occur widely in human foodstuffs, both as intentional additives and as undesirable contaminants (Fytianos and Zarogiannis, 1999). Human dietary nitrate and nitrite exposure should be controlled as they may be considered a health risk factor. Vegetables constitute the major dietary source of nitrates generally providing more than 80% of the daily dietary intake (Zhou *et al*., 2000). Much of nitrate in vegetables is naturally occurring and is influenced by factors including plant species and variety, light intensity, temperature, fertilizing (Tosun and Ustun, 2004). Nitrite concentrations are usually very low in fresh vegetables but under adverse post-harvest storage conditions nitrite levels can increase in vegetables as a result of bacterial or endogenous nitrate reductase reducing the nitrate to nitrite (Hunt and Turner, 1994; Mendicoa, 1997).

The significance of nitrate to human health derives from the fact that nitrate can be converted *in vivo* to nitrite which interacts with hemoglobin to affect the oxygen transport mechanism giving rise to a condition known as methaemoglobinaemia (Meah *et al*., 1994). Furthermore, the toxic effects of nitrite have been reviewed in many studies (Chung *et al*., 2003; Concon, 1988; Meah *et al*., 1994).

The Acceptable Daily Intake (ADI) for nitrate of 3.7 mg nitrate kg^{-1} body weight was established by EU Scientific Committee for Food in 1995. Nitrite has much higher acute toxicity than nitrate and a lower ADI of 0.06 mg nitrite kg^{-1} body weight (EU Scientific Committee for Food 1995; Petersen and Stoltze, 1999). This corresponds to 256 and 4.2 mg nitrite day^{-1} for a person weighing 70 kg.

Concentrations of nitrate and nitrite in vegetables have been the focus of attention in several countries (Chung *et al*., 2003; Fytianos and Zarogiannis, 1999; Meah *et al*., 1994; Petersen and Stoltze, 1999). However, nitrate and nitrite in vegetables have not been well studied in Turkey.

Turkey is a developing county and agriculture plays a very important role its economic development. The utilization of nitrogenous fertilizers has become a public interest in the last decade. The objective of this study was to investigate the content of nitrate and nitrite in vegetables mostly consumed by Turkish people and to evaluate the overall safety of natural nitrates and nitrites in vegetables.

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MATERIALS AND METHODS

The samples were collected from wholesalers of fruit and vegetable markets in Ankara in Turkey. 258 samples of 7 different vegetables (tomato, carrot, butterhead lettuce, cripshead lettuce, iceberg, parsley and spinach) were collected through January 2001-April 2002. After purchase, samples were transported to the laboratory in cool boxes. Prior to analyses non-edible parts of the samples were removed and washed with distilled water.

Dry matter contents of the samples were determined by oven-drying at 105°C until a constant weight was obtained (AOAC, 1990). Nitrate and nitrite were determined spectrophotometrically according to the method of ISO (1984). The method involves dissolution of vegetables in warm water, precipitation of the fat and proteins and filtration. Nitrate was reduced to nitrite in a portion of the filtrate by means of metallic cadmium in a glass column. A red color was developed in portions of both reduced solution and unreduced filtrate by the addition of sulfanilamide and N-1-naphtyl-ethylenediamide. Measurement of the color intensity was made by spectrophotometer at a wavelength of 538 nm and compared with standard nitrite solutions. Calculation of the nitrate content was made from the difference between these two analytical results. All estimations were carried out in duplicate. The method was continuously tested by standard addition of nitrates and nitrites in each type of vegetables studied. Recoveries have been found to be between the 98 and 102%. The limit of detection was 5 mg kg^{-1} for nitrate and 0.3 mg kg^{-1} for nitrite (ISO, 1984; Petersen and Stoltze, 1999).

RESULTS AND DISCUSSION

The range of dry matter contents and the nitrate concentrations of each vegetable samples were presented in Table 1 and 2, respectively. The highest amount of nitrate values was observed in leafy vegetables. The leafy vegetables, parsley contained the highest amount of nitrate (1513.36 mg kg^{-1}) followed by spinach (1456.04 mg kg^{-1}), cripshead lettuce (1042.81 mg kg^{-1}), butterhead lettuce (914.22 mg kg^{-1}), iceberg (623.38 mg kg^{-1}). Tomato (11.06 mg kg^{-1}) had the lowest levels of nitrate. Carrot (190.03 mg kg^{-1}) contained also low nitrate concentration. The nitrate concentrations of vegetables were determined under the levels established by the European Commission (max 2500 mg nitrate kg^{-1} fresh weight) (Petersen and Stoltze, 1999).

The widest range in nitrate concentration was found in parsley and spinach and the smallest in tomato and carrot. As it was seen in Table 2, great variation in the content of nitrate was found for all samples. Differences in nitrate accumulation may due to harvesting period, amount and kind of nutrients present in soil, amount and composition of the fertilizers applied, temperature and light factors from the ecological environment (Tosun and Ustun, 2004; Zhou *et al.*, 2000).

Table 1: Dry matter contents of vegetables (%)

Vegetables	n	Range	Mean	SD
Tomato	36	2.92-7.49	5.28	0.90
Carrot	36	6.40-11.43	9.86	1.01
Butterhead lettuce	36	3.36-6.22	4.87	0.70
Cripshead lettuce	36	3.47-5.74	4.75	0.52
Iceberg	36	2.59-5.32	4.04	0.57
Parsley	36	7.98-14.45	10.48	1.36
Spinach	42	5.37-11.86	7.45	1.72

N: Numbers of the samples; SD: Standard Deviation

Table 2: Nitrate contents of vegetables (mg kg^{-1} fresh weight)

Vegetables	n	Range	Mean	SD
Tomato	36	ND-71.00	11.06	13.340
Carrot	36	8.06-509.44	190.03	131.97
Butterhead lettuce	36	280.96-1872.51	914.22	384.79
Cripshead lettuce	36	431.31-1867.93	1042.81	359.22
Iceberg	36	295.30-1708.88	623.38	244.55
Parsley	36	136.76-3136.71	1513.36	804.11
Spinach	42	29.32-2478.26	1456.04	658.13

N: Numbers of the samples; SD: Standard Deviation; ND: Not Detected

Table 3: Nitrite contents of vegetables (mg kg^{-1} fresh weight)

Vegetables	n	Range	Mean	SD
Tomato	36	ND-1.33	0.36	0.26
Carrot	36	ND-1.79	0.65	0.35
Butterhead lettuce	36	ND-1.69	0.84	0.37
Cripshead lettuce	36	ND-2.12	0.98	0.47
Iceberg	36	ND-1.85	0.92	0.44
Parsley	36	ND-7.60	1.78	1.41
Spinach	42	ND-12.11	2.31	2.63

N: Numbers of the samples; SD: Standard Deviation; ND: Not Detected

Normally small amounts of nitrite are present in vegetables but nitrite can be formed due to reduction of nitrate if the vegetables are stored incorrectly. This is especially a problem in leafy vegetables for which soil may be difficult to remove completely (Abo Bakr and El-Iraqui, 1986; Teotia *et al.*, 1988). Nitrite contents of samples were given in Table 3. There were large variations in the amount of nitrite found in spinach and parsley. The mean nitrite values of spinach, parsley, iceberg, cripshead lettuce, butterhead lettuce, carrot and tomato were determined as 2.31, 1.78, 0.92, 0.98, 0.84, 0.65 and 0.36 mg kg^{-1} in fresh weight, respectively. Based on these data, nitrite concentrations in vegetables in Turkey are low and of little concerns.

In general, the leafy vegetables had higher nitrite contents than root and fruit vegetables. Our results are in agreement with those reported by other investigators who stated that the nitrite concentrations in fresh vegetables were usually very low (Abo Bakr and El-Iraqui, 1986; Chung *et al.*, 2003; Petersen and Stoltze, 1999).

The nitrate concentrations of lettuce samples were lower than those from earlier study in Turkey (Tosun and Ustun, 2004). The results of all samples are in agreement with Erkmen *et al.* (1990). The vegetables harvested in Turkey showed lower levels of nitrate and nitrites compared with those levels monitored elsewhere. The average nitrate levels of each vegetable varied depending on the country (EU Scientific Committee for Food, 1995) the average levels of nitrate of lettuce and spinach were lower than those in Korea, Italy, China and Denmark. Monitoring results from UK (MAFF, 1996) showed that nitrate contents were similar in tomato, parsley, carrot and iceberg but lower in spinach and lettuce.

This study has produced results indicating that the means of nitrate and nitrite in vegetables are similar to those reported by EU and some of the data were even found to be lower than those of the EU, implying the non-toxicity of these vegetables.

The nitrate and nitrite values of vegetables having high consumption in Turkey as reported in this study do not necessarily pose any health hazard to the consumer under normal conditions. However, frequent consumption of large quantities of vegetables high in nitrate contents may prove hazardous, particularly to infants.

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