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Nitrate and Nitrite Accumulation in Fresh Vegetables from Greece

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Concentrations of nitrate and nitrite in vegetables have been the focus of attention in several countries. Nitrate and nitrite occur widely in human and animal foodstuffs, both as intentional additives and as undersirable contaminants. It has long been recognised that high levels of these compounds are undesirable in certain foodstuffs such as baby foods. The interest in their accurate determination at relatively low levels has been heightened in the last decade with the upsurge of interest in the formation of N-nitrosa compounds (Usher et al., 1975).

Naturally occuring nitrates in fresh foods and in water supplies make a substantially greater contribution to the total dietary intake of most consumers. Nitrates are present in all plants and are an essential source of nitrogen for normal growth. It is inescapable, therefore, that foods of plant origin will contain some nitrate and frequently substantial amounts (Walker et al. 1975, Lyons et al. 1994, Siciliano et al. 1975).

Many studies have demonstrated consistently that the leaf, stem and flower vegetables are generally rich in nitrate (Kenny et al. 1975, Maynard et al. 1972). It is widely known that spinach contains particularly high nitrate concentrations, commonly around 1000 ppm, and values in excess of 3000 ppm have been reported on several occasions. It is less widely known that lettuce is similarly rich in nitrate and concentrations approaching 6000 ppm have been reported. Artichoke, cabbage, cauliflower, celery, kale and leek usually contain nitrate at concentrations ranging from several hundreds to over 1000 ppm, while concentrations in asparagus, chicory and onions are rather lower.

The equilibrium of nitrate concentration in vegetables depends mainly on the rate at which nitrate is taken up by the plant from the soil and the activity of endogenous nitrate reductase. There is ample evidence that, in a wide range of plant species, application of nitrogenous fertilisers leads to accumulation of nitrate (Barker et al. 1971). Most of the nitrogen (N)

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in plants is taken up initially in the nitrate (NO_3) form. Other factors which influence plant nitrate levels, to a lesser extent, are cultivar, source of N, macronutrients other than N and light conditions (Cantiliffe, 1973). Accumulation of nitrate also varies with plant organs. Stems, petioles, leaves, roots, flowers and fruits generally show a decreasing tendency in that order to accumulate nitrate.

Nitrite concentrations in fresh, undamaged plant tissues are usually very low and it appears that under normal growing conditions the rate of reduction of endogenous nitrite matches that at which nitrate is reduced to nitrite (Wright et al.). However, vegetables which accumulate very high concentrations of nitrate may also contain significant amounts of nitrite. Post-harvest storage of vegetables certainly can lead to the accumulation of high nitrite concentrations which arises mainly from microbial reduction of nitrate.

There are many methods available for both qualitative and quantitative determination of nitrates in foods (Lyons et al. 1991, Schuster et al. 1987, Singh 1988, Smith 1975).

In the present study an improved calorimetric method according to Sen et al. (1978) is applied. The concentration of nitrate is determined after reduction to nitrite on a cadmium column.

MATERIALS AND METHODS

Samples of fresh vegetables from Greece were analysed for nitrite and nitrate.

An aliquot of 10 g of well homogenized sample is blended for 5 min with 70 ml water and 12 ml 12% NaOH (pH = 8). The solution is transferred to a 200 ml volumetric flask and heated in water bath (50-60°C with occasional swirling until temperature of suspension is ca. 50°C). After cooling the extracts were filtered before analysis through Whatman No 41 filter-papers. The first zone of filtrate was discarded in order to overcome possible nitrate contamination from the filter-papers.

An aliquot of 10 ml filtrate is mixed with 5.0 ml NH_4Cl buffer and passed through the Cd column, The nitrite is determined calorimetrically by diazotization of sulfanilamide and subsequent coupling with N-(l-napthyl)-ethylenediamine to form a pink azodye whose absorbance is measured at 543 nm against aqueous prepared standards.

To obtain nitrite level resulting from nitrate, free nitrite value was subtracted from the total nitrite value (nitrite originally present in the sample + nitrite formed from nitrate by reduction).

Recovery of NO_3 from vegetables: From each commodity recovery tests were performed in which different amounts of NO_3 were added before analysis. Table 1 lists the results of recovery addition of nitrate to vegetables samples.

Table 1. Recovery of nitrate added to vegetables

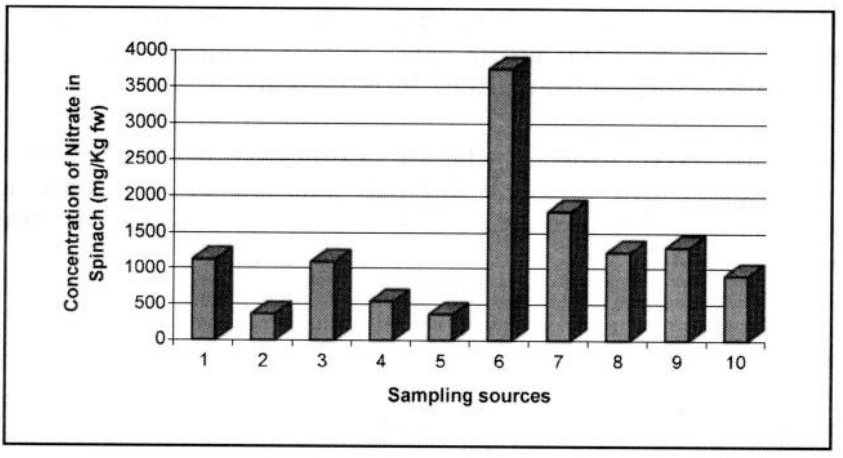
Sample	Originally Present	Added	Total found	Recovery %
Cabbage	194	200	360	91.4
Cellery	209	250	389	84.7
Cucumber	91	150	204	84.8
Leek	107	150	205	80.0
Lettuce	175	300	457	96.2
Onion, whole	53.7	120	138	79.4
Spinach	1560	1200	3044	110
Tomato	10.3	40	42.7	84.9

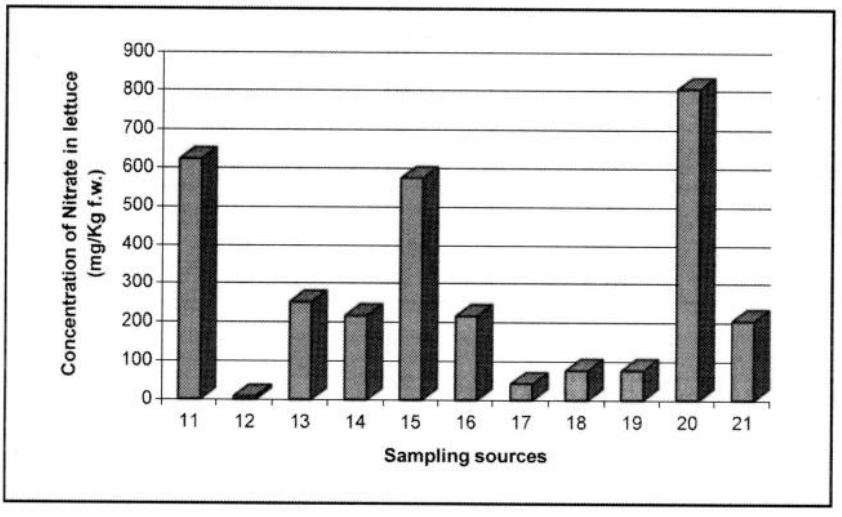
RESULTS AND DISCUSSION

Nitrite content: All samples were tested for nitrite, but it was detected only in some samples of the examined vegetables. Nitrite concentrations were mainly detected in spinach from 0.8 to 8.5 ppm (mean value 4.0 ppm). It was also detected in some only samples of cabbage, lettuce and celery (from 0.06 to 0.23 for cabbage, from 0.15 to 0.32 for lettuce and from 0.15 to 0.41 ppm for cucumber). In the samples of leek, onions and tomato no nitrite concentrations were detected.

Based on these data, nitrite concentrations in vegetables from Greece are low and of little concern.

Nitrate content: In contrast, nitrate was detected almost in all vegetables at widely varying concentrations. Differences in nitrate accumulation may be related to differences in uptake, assimilation or translocation (Maynard et al. 1976).





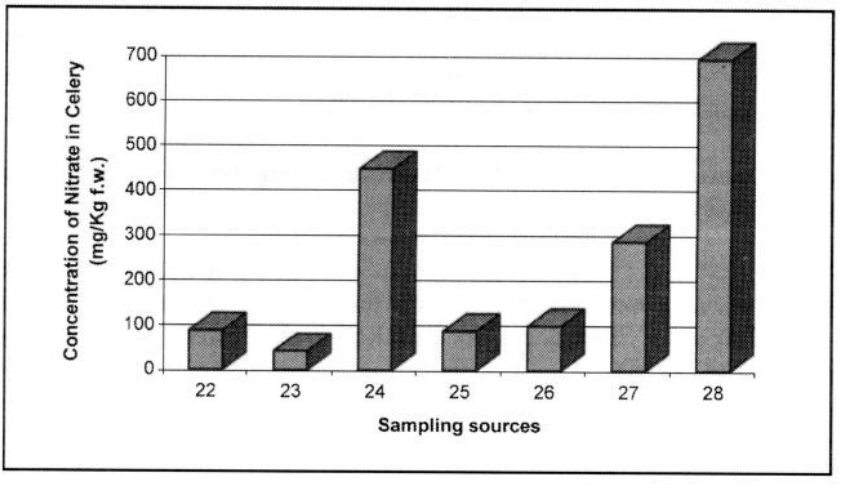


Figure 1. Concentration of nitrate in vegetables from different sampling sources

The high variation in nitrate levels for the same vegetable between different regions (Fig. 1) was not surprising as nitrate levels in plant parts are very sensitive to changes in N supply.

In 32% of the examined samples, NO_3 and NO_2 were detected, in 39% of the samples (only NO_3 was detected) in 7% of them only NO_2 and in 22% neither NO_3 nor NO_2 were detected.

Results of analysis of eight fresh vegetables from different places of Greece available in super markets are given in Table 2. From the examined vegetables, spinach contained the highest level of nitrate (mean value 1.252 ppm). Lettuce, celery and cabbage contained also high nitrate concentrations, while concentrations in cucumber, leek, onions and tomato were lower. The mean value was estimated taking into account only the samples that were positive to NO_3 detection.

Taking a recommended upper limit of 300 ppm NO_3-N (1330 ppm NO_3), the nitrate levels found in some samples of spinach would render these samples unsafe for use in feeding infants under 3 months.

Generally, the nitrate concentrations found in the examined vegetables are similar to values in the literature (Maynard et al. 1976).

Table 2. Nitrate content ($mg.kg^{-1}$ fresh weight) of processed vegetables

Vegetable	No of samples from different sources	Nitrate		
		Range	Mean	SD
Cabbage	10	19.6 - 414	209	243
Celery	7	43 - 692	250	242
Cucumber	10	19.5 - 576	157	188
Leek	10	42.6 - 333	132	124
Lettuce	12	8.0 - 808	282	266
Onions, whole	9	19.8 - 240	127	84
Spinach	10	545 - 3760	1250	989
Tomato	9	8.2 - 54.6	34.2	19.8

In conclusion, this study,though limited in scope, serves to indicate that nitrate levels in vegetables with a high consumption in Greece, are relatively low and safe, while the levels in some spinach, lettuce and celery are high enough to warrant periodic monitoring and, possibly, further investigation.

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