# Contribution of different vegetable types to exogenous nitrate and nitrite exposure

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ABSTRACT

This study reports the levels of nitrate and nitrite of 34 vegetable samples, including different varieties of cabbage, lettuce, spinaches, parsley and turnips, collected in several locations of an intensive agricultural area (Modivas, Vila do Conde, northern Portugal). Nitrate levels ranged between 54 and 2440 mg $NO_3^-$ kg$^{-1}$, while nitrite levels ranged between 1.1 and 57 mg $NO_2^-$ kg$^{-1}$. The maximum residue levels established for nitrate in spinach and lettuce samples were not exceeded. Nitrate and nitrite levels reported in the literature for the same type of samples are reviewed, as well as the contribution of vegetables to nitrate and nitrite dietary exposure of populations.

Keywords: Nitrate, Nitrite, Vegetables, Dietary exposure

## 1. Introduction

Nitrate and nitrite are natural constituents of plant material. Although nitrate is apparently non-toxic below maximum residue levels (MRLs), it may be endogenously transformed to nitrite which can react with amines and amides to produce *N*-nitroso compounds (Santamaria, 2006; Yordanov, Novakova, & Lubenova, 2001). These have been related to an increased risk of gastric, oesophageal, nasopharyngeal and bladder cancers (Choi, Chung, Lee, Shin, & Sung, 2007). For this reason the determination of nitrate and nitrite in food has received increased attention and several recent studies have addressed the dietary exposure of human population to these compounds (Menard, Heraud, Volatier, & Leblanc, 2008; Thomson, Nokes, & Cressey, 2007).

According to the literature, the primary dietary sources of nitrate and nitrite include vegetables, processed and cured meat, fish and poultry (to which nitrite has been added), and possibly water, especially if there is runoff or contamination by nitrate from agricultural sources. Plant foods are the primary sources of nitrate (80–95%), while processed and cured meat are the primary sources of nitrite (Pennington, 1998; Ximenes, Rath, & Reyes, 2000).

About 5% of the dietary nitrate is reduced to nitrite in saliva and the gastrointestinal tract. This value can reach 20% for individuals with a high rate of conversion (Thomson et al., 2007). Methemoglobinemia is another health hazard attributed to nitrite, a condition where reduced iron ($Fe^{2+}$) in haemoglobin is oxidised by nitrite to $Fe^{3+}$, thus reducing the total oxygen-carrying capacity of the blood (Santamaria, 2006).

Nitrate concentrations in vegetables depend on the biological properties of the plant culture, (day)light intensity, type of soil, temperature, humidity, frequency of plants in the field, plant maturity, vegetation period, harvesting time, size of the vegetable unit, storage time and source of nitrogen (Tamme et al., 2006). Nitrogen fertilisation and light intensity have been identified as the major factors that influence nitrate content in vegetables (Gruda, 2005; Santamaria, 2006). Even among different samples of the same vegetable varieties, the range of concentrations may be large (Prasad & Chetty, 2008; Tamme et al., 2006; Thomson et al., 2007). Variations, within site, for plants growing in the same plot can be explained by differences in soil mineral nitrogen, and individual plant growth in response to localised areas of soil compaction, or variability in soil pH (Weightman, Dyer, Buxton, & Farrington, 2006).

Generally, nitrate-accumulating vegetables belong to the families of Brassicaceae (rocket, radish, mustard), Chenopodiaceae (beetroot, Swiss chard, spinach) and Amarantaceae; but also Asteraceae (lettuce) and Apiaceae (celery, parsley) include species with high nitrate contents (Santamaria, 2006). The differing locations of the nitrate reductase activity, as well as the differing degrees of nitrate absorption and transfer in the plant may be responsible for the varying capacities of vegetable species to accumulate nitrate (Santamaria, 2006).

Although vegetables are one of the major sources of dietary exposure to nitrate, numerous studies have reported that the consumption of vegetables decreases in incidences of cancer. This is mainly due to antioxidant compounds (ascorbate, tocopherol, b-carotene, phenol compounds, indol) that suppress the formation of carcinogenic agents, such as nitrosamines (Chung et al., 2003). Therefore, it has been argued that naturally occurring nitrates in foods are not toxic, which is one of the reasons for the absence of regulations controlling nitrates in vegetables in some countries (Chung et al., 2003). The European Commission has established maximum levels (MLs) for nitrate in lettuces (2500–3500 mg $kg^{-1}$ for summer harvest and 4000–4500 mg $kg^{-1}$ for winter harvest) and spinaches (2500 mg $kg^{-1}$ for summer harvest, 3000 mg $kg^{-1}$ for winter harvest and 2000 mg $kg^{-1}$ for frozen spinach) (European Commission Regulation (EC) No 1881/2006). In China, a suggested maximum level of nitrate in vegetables of 3100 mg $kg^{-1}$ has been established (Santamaria, 2006) and the tolerance limit for nitrite is 4 mg $kg^{-1}$ (Zhou, Wang, & Wang, 2000).

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 defence (Santamaria, 2006).

The Vila do Conde region, northern Portugal, is classified as a vulnerable area because of the high nitrate concentrations that may be found in water and soil samples, as a consequence of intensive agricultural activity (Gonçalves, Esteves da Silva, & Alpendurada, 2006). The evaluation of the nitrate and nitrite levels in horticultural products grown in this area is therefore important in order to assess their safety for consumers. These products play an important role in the population's diet, being consumed on a daily basis, especially in the form of soup. In this study, the levels of nitrate and nitrite were determined in 34 vegetable samples, consisting of different varieties of cabbage, lettuce, spinach, parsley and turnip, collected in Vila do Conde, northern Portugal.

## 2. Materials and methods

### 2.1. Chemicals

All reagents used in this study were of analytical grade. De-ionised water (18 M X $cm^{-1}$) was used for preparing all the solutions and for sample extraction. Potassium nitrite (Riedel-de Häen), potassium nitrate (Riedel-de Häen), sulphanilamide chloride (Sigma–Aldrich), *N*-(1-naphthyl)ethylenediamine dihydrochloride (Riedel-de Häen), potassium hexacyanoferrate (II) (Riedel-de Häen), ammonium chloride (Merck), hydrochloric acid (Riedel-de Häen), zinc acetate (Fluka), disodium tetraborate (Merck) and glacial acetic acid (Merck) were used.

### 2.2. Vegetable samples

In total, 34 vegetable samples (Portuguese cabbage (*Brassica oleracea* var. *costata*), kale (*B. oleracea* var. *acephala*), turnip and turnip sprouts ("grelos") (*Brassica rapa* var. *rapa*), lettuce (*Lactuca sativa*), spinach (*Spinacea oleracea*), and parsley (*Petroselinum crispum*)), from several different producers, were collected in different locations of Modivas, Vila do Conde municipality, in February, 2007. The vegetable samples were labelled and frozen in plastic bags after collection. Just before analysis they were cut into small pieces and macerated in a blender.

### 2.3. Extraction and analysis

Nitrate and nitrite concentrations were determined using a standard methodology (ISO 6635:1984 (E)). Briefly, the method consists in extracting a test portion of the vegetable (10 g) with hot water, precipitating the proteins by addition of solutions of potassium hexacyanoferrate (II) and zinc acetate, and filtering the precipitate. The nitrate is then reduced to nitrite in a column containing metallic cadmium. The addition of sulphanilamide chloride and *N*-(1-naphthyl)ethylenediamine to the nitrite-containing solutions allows the measurement of the red complex obtained, at 538 nm. The limit of detection (LOD) was 0.093 mg $NO_2^-$ $kg^{-1}$ while the limit of quantification (LOQ) was 0.31 mg $NO_2^-$ $kg^{-1}$.

## 3. Results and discussion

As was pointed out by Menard et al. (2008), although the value of national monitoring programmes is unquestionable, these programmes do not account for the variability of food composition across regions. Therefore it is important to perform studies addressing areas and regions where the levels of nitrate and/or nitrite may be potentially higher. Regarding the production of fresh vegetables in northern Portugal, Vila do Conde is an important region. Cultures are grown both in the open air and greenhouses and large quantities of fertilisers are used. A study conducted in 2005 revealed that 74% of groundwater samples obtained from domestic wells, with an approximate depth of 15 m, presented nitrate concentrations higher than the Portuguese guideline value for drinking water (50 mg $l^{-1}$) (Silva et al., 2006). Furthermore, 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 and nitrite levels on the studied samples are presented in Table 1, while average contents are shown in Table 2.

The results obtained show a considerable variation in the nitrate contents within the same vegetable species. As previously described, the nitrate content in vegetables depends on many factors, such as soil properties, fertiliser usage, cultivation and weather conditions, which are unknown and whose effects are impossible to account for in this study. Considering all these different factors, wide ranges and large standard deviations may occur (Pennington, 1998).

Regarding the nitrate content, 82% of the samples presented nitrate levels lower than 1000 mg $kg^{-1}$. The average nitrate contents for Portuguese cabbage and kale (547 and 472 mg $kg^{-1}$, respectively) were similar and comparable to the results obtained for cabbages in other countries (Table 3), particularly those reported in Estonia (437 mg $kg^{-1}$) and France (498 mg $kg^{-1}$) (Menard et al., 2008; Tamme et al., 2006).

For turnip sprouts ("Grelos"), the nitrate mean value was 489 mg $kg^{-1}$ while, for the two turnip samples, the average value was 444 mg $kg^{-1}$. Comparing with the nitrate contents presented in Table 3, these are in the range of those found for turnip in Estonia and France (307 and 657 mg $kg^{-1}$, respectively) and are lower than those reported in Brazil and China (2098 and 2127 mg $kg^{-1}$, respectively) (Ximenes et al., 2000; Zhou et al., 2000).

In our study, the highest nitrate value was obtained for a parsley sample (2440 mg $kg^{-1}$). According to the literature, typical nitrate contents in parsley may be considered high, and are usually in the range 1000–2500 mg $kg^{-1}$ (Santamaria, 2006).

The maximum residue levels established for nitrate in spinach and lettuce samples were not exceeded but the mean values, 1112 and 1156 mg $kg^{-1}$, respectively, were the highest of all the analysed samples (Table 2).

With the exception of lettuce, the vegetables considered in this study are usually consumed after cooking, either after boiling or included in soups. Cooking vegetables has been shown to reduce nitrate concentrations by up to 75% (Menard et al., 2008). In the first case, the boiling water is usually discarded and therefore a reduction in nitrate levels is expected. In regard to the soups, although

Table 1
Nitrate and nitrite contents (mg kg$^{-1}$) in the analysed vegetable samples.

| Vegetable sample | Nitrate (mg kg$^{-1}$) | Nitrite (mg kg$^{-1}$) |
|---|---|---|
| Portuguese cabbage 1 | 41 | 1.4 |
| Portuguese cabbage 2 | 162 | 30.0 |
| Portuguese cabbage 3 | 336 | 1.9 |
| Portuguese cabbage 4 | 405 | 20.5 |
| Portuguese cabbage 5 | 643 | 1.9 |
| Portuguese cabbage 6 | 733 | 0.9 |
| Portuguese cabbage 7 | 804 | 17.3 |
| Portuguese cabbage 8 | 863 | 9.1 |
| Portuguese cabbage 9 | 939 | 3.7 |
| Turnip sprouts 10 | 54 | 1.3 |
| Turnip sprouts 2 | 178 | 1.1 |
| Turnip sprouts 9 | 305 | 1.3 |
| Turnip sprouts 5 | 349 | 39.8 |
| Turnip sprouts 6 | 364 | 1.8 |
| Turnip sprouts 1 | 728 | 1.1 |
| Turnip sprouts 4 | 1447 | 57.0 |
| Kale 8-1 | 41 | 4.4 |
| Kale 10 | 90 | 2.9 |
| Kale 8-2 | 180 | 2.3 |
| Kale 6 | 288 | 3.1 |
| Kale 9 | 572 | 4.1 |
| Kale 7 | 811 | 1.4 |
| Kale 11 | 1319 | 1.2 |
| Colza 8 | 73 | 2.2 |
| Spinach 8 | 797 | 13.8 |
| Spinach 11 | 1427 | 5.2 |
| Lettuce 6 | 1156 | 2.6 |
| Parslev 12 | 9 | 1.3 |
| Parsley 3-1 | 136 | 3.0 |
| Parsley 4 | 866 | 2.1 |
| Parsley 3-2 | 1006 | 11.0 |
| Parsley 7 | 2441 | 13.4 |
| Turnip 10 | 234 | 1.1 |
| Turnip 9 | 654 | 1.4 |

Numbers refer to the collection site.

there is a reduction in nitrate content in the vegetable tissue, nitrates are transferred to the liquid phase. In fact the determination of nitrates in vegetable samples, in many analytical methods, is based on nitrate extraction from test portions with hot water. In the final soup, nitrate concentration may be increased if the water used in the preparation has a high nitrate content. In the region considered in this study this possibility exists, since the water supply is not available in all the households. Another "common" idea is that boiling increases water safety, which may be true in the case of microbiological contamination but, in the case of nitrate, will have an opposite effect as an increase in nitrate concentration may occur due to water evaporation.

According to the literature, vegetables were shown to contain nitrates at varying levels, ranging from 1 to 10,000 mg kg$^{-1}$. A value of 10,800 mg kg$^{-1}$ has been reported for a celery sample grown using the hydroponic cultivation method (Zhong, Hu, & Wang, 2002).

The time of the day the plants were harvested was shown to have a significant effect on the variability of nitrate levels in lettuce, although with no consistent temporal trends (Weightman et al., 2006). However other studies failed to detect a significant relationship between nitrate levels and time of harvest within the day (Reinink, 1991).

Nitrate contents of organic and conventional vegetables may differ significantly. In Belgium, the mean nitrate contents of organic and conventional products were significantly different, 1703 and 2637 mg kg$^{-1}$, respectively (Pussemier, Larondelle, Van Peteghem, & Huyghebaert, 2006). On the other hand, in a study conducted in Italy, organically grown vegetables contained significantly higher contents of nitrate than did conventionally cultivated products (De Martin & Restani, 2003).

Regarding the nitrite contents, four samples (two turnip sprouts "Grelos" and two Portuguese cabbages) presented nitrite levels >20 mg kg$^{-1}$ (Table 1). These values are higher than those that are usually reported in fresh vegetable samples. It is commonly assumed that the nitrite levels in fresh leafy vegetables are usually less than 2 mg kg$^{-1}$ (Santamaria, 2006). In this study, nitrite levels were lower than 5 mg kg$^{-1}$ in 70% of the analysed samples and lower than 20 mg kg$^{-1}$ in 90% of the total.

It has been shown that nitrite concentrations in fresh, uninjured, well-stored vegetables are extremely low, possibly because the nitrite reductase activity rate is in equilibrium with one of the nitrate reductase enzymes under proper storage conditions (Chung, Chou, & Hwang, 2004). Nitrite concentrations may increase dramatically via microbiological reduction of nitrate in vegetables while the nitrate content decreases during storage at ambient temperature. Under refrigerated storage, however, nitrite accumulation tends to be inhibited but may take place (Chung et al., 2004; Prasad & Chetty, 2008). Under frozen storage, nitrite accumulation is inhibited. Therefore, a poor storage could probably

Table 2
Nitrate and nitrite mean contents (mg kg$^{-1}$) for the different vegetable samples.

| Vegetable (common name and specie) | | Nitrate (mg kg$^{-1}$) | | | | Nitrite (mg kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | Min | Max | Mean | SD | Min | Max | Mean | SD |
| Portuguese cabbage *Brassica oleracea* var. *costata* | 9 | 41 | 939 | 547 | 323 | 0.8 | 30 | 9.6 | 10.6 |
| Turnip sprouts ("grelos") *Brassica rapa* var. *rapa* | 7 | 54 | 1447 | 489 | 471 | 1.1 | 57 | 14.8 | 23.5 |
| Kale ("Couve galega") *Brassica oleracea* var. *acephala* | 7 | 41 | 1319 | 472 | 464 | 1.2 | 4.4 | 2.8 | 1.2 |
| Parsley *Petroselinum crispum* | 5 | 9 | 2440 | 891 | 970 | 1.3 | 13.4 | 6.2 | 5.6 |
| Spinach *Spinacea oleracea* | 2 | 797 | 1427 | 1112 | | 5.2 | 13.8 | 9.5 | |
| Turnip (root) *Brassica rapa* var. *rapa* | 2 | 234 | 654 | 444 | | 1.1 | 1.4 | 1.3 | |
| Letuce *Lactuca sativa* | 1 | | | 1156 | | | | 2.6 | |
| Colza ("Couve-nabiça") *Brassica napus* | 1 | | | 73 | | | | 2.2 | |

n: number of samples; SD: Standard deviation.

Table 3
Nitrate contents (mg kg$^{-1}$) in fresh vegetables (cabbage, lettuce, parsley, spinach and turnip) described in some recent studies.

| Vegetable | Year reported | Author | Country | Nitrate (mg kg$^{-1}$) | | | Number of samples | Notes |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | Minimum | Maximum | | |
| Cabbage | 1999 | Fytianos and Zarogiannis (1999) | Greece | 209 | 19.6 | 414 | 10 | |
| | 2002 | Zhong et al. (2002) | China | 1530 | 26 | 2670 | | North China (1998–1999) |
| | 2003 | Chung et al. (2003) | Korea | 730 | 29 | 1498 | 15 | Winter (November–March), 1998 |
| | | | | 722 | 1 | 1788 | 25 | Summer (April–October), 1998 |
| | 2006 | Tamme et al. (2006) | Estonia | 437 | 74 | 1138 | 168 | (2003–2004)[a] |
| | 2006 | Sušin, Kmecl, and Gregorc˘ic (2006) | Slovenia | 881 | 112 | 1864 | 52 | (1996–2002)[a] |
| | 2007 | Thomson et al. (2007) | New Zealand | 241 | 88 | 503 | 8 | November–December, 2003; 2 urban regions |
| | 2008 | Menard et al. (2008) | France | 498 | – | 1855 | 16 | Green cabbage (2000–2006)[a]; 6.3% of the samples < LOD |
| | 2008 | Prasad and Chetty (2008) | Fiji | 1425 | | | 6 | "English cabbage", SD = 732 mg kg$^{-1}$ (2006) |
| Lettuce | 1999 | Fytianos and Zarogiannis (1999) | Greece | 282 | 8 | 808 | 12 | |
| | 2000 | Ximenes et al. (2000) | Brazil | 1420 | – | – | 1 | |
| | 2000 | Zhou et al. (2000) | China | 896 | 580 | 1454 | 6 | Beijing City (1979–1981) |
| | 2003 | Chung et al. (2003) | Korea | 1933 | 247 | 3283 | 15 | Winter (November–March), 1998 |
| | | | | 2728 | 884 | 4488 | 25 | Summer (April–October), 1998 |
| | 2003 | De Martin and Restani (2003) | Italy | 1473 | <LOD | 3270 | 177 | (1996–2002) |
| | 2006 | Tamme et al. (2006) | Estonia | 2167 | 397 | 3230 | 14 | (2003–2004)[a] |
| | 2006 | Sušin et al. (2006) | Slovenia | 1074 | 21 | 3986 | 151 | (1996–2002)[a] |
| | 2006 | Weightman et al. (2006) | UK | 838 | – | – | 60 | Goodison var., open field, September 2003 |
| | | | | 1352 | – | – | 60 | Iceberg var., open field, September 2003 |
| | 2006 | Merino, Darnerud, Edberg, Åman, and Castillo (2006) | Sweden | 2684[b] | 58 | 5406 | 159 | Fresh lettuce, under covered fields (1996–2005)[a] |
| | | | | 826[b] | 442 | 2038 | 14 | Fresh lettuce, organic farming (1996–2005)[a] |
| | | | | 931[b] | 94 | 2298 | 71 | Iceberg lettuce (1996–2005)[a] |
| | 2007 | Thomson et al. (2007) | New Zealand | 1160 | 61 | 2495 | 18 | November–December, 2003; 2 urban regions |
| | 2008 | Menard et al. (2008) | France | 1974 | – | 5600 | 579 | (2000–2006)[a]; 1.9% of the samples < LOD |
| | 2008 | Prasad and Chetty (2008) | Fiji | 1297 | | | 6 | SD = 348 mg kg$^{-1}$ (2006) |
| Parsley | 2002 | Öztekin, Nutku, and Erim (2002) | Turkey | 1204 | 1204 | 1204 | 1 | |
| | 2006 | Tamme et al. (2006) | Estonia | 966 | 674 | 1588 | 4 | (2003–2004)[a] |
| | 2008 | Menard et al. (2008) | France | 1980 | – | 4300 | 16 | (2000–2006)[a]; 6.3% of the samples <LOD |
| Spinach | 1999 | Fytianos and Zarogiannis (1999) | Greece | 1250 | 545 | 3760 | 10 | |
| | 2000 | Ximenes et al. (2000) | Brazil | 528 | – | – | 1 | |
| | 2000 | Zhou et al. (2000) | China | 2358 | 239 | 3872 | 35 | Beijing City (1979–1981) |
| | 2002 | Öztekin et al. (2002) | Turkey | 2820 | 2820 | 2820 | 1 | |
| | 2003 | Chung et al. (2003) | Korea | 3334 | 427 | 7439 | 15 | Winter (November–March), 1998 |
| | | | | 4814 | 195 | 7793 | 25 | Summer (April–October), 1998 |
| | 2003 | De Martin and Restani (2003) | Italy | 1757 | <LOD | 3720 | 23 | (1996–2002) |
| | 2005 | Jaworska (2005a) | Poland | 900 | | | | SD = 264 mg kg$^{-1}$; New Zealand spinach |
| | 2006 | Tamme et al. (2006) | Estonia | 2508 | 2508 | 2508 | 1 | (2003–2004)[a] |
| | 2006 | Merino et al. (2006) | Sweden | 1747[b] | 47 | 5975 | 63 | Fresh spinach (1996–2005)[a] |
| | 2007 | Thomson et al. (2007) | New Zealand | 722 | 73 | 1138 | 8 | November–December, 2003; 2 urban regions |
| | 2008 | Menard et al. (2008) | France | 1682 | – | 8700 | 266 | (2000–2006)[a]; 3.4% of the samples <LOD |
| Turnip | 2000 | Ximenes et al. (2000) | Brazil | 2098 | – | – | 1 | |
| | 2000 | Zhou et al. (2000) | China | 2127 | | | 1 | Beijing City (1979–1981) |
| | 2006 | Tamme et al. (2006) | Estonia | 307 | 64 | 1062 | 58 | (2003–2004)[a] |
| | 2008 | Menard et al. (2008) | France | 657 | – | 2128 | 42 | (2000–2006)[a] |

SD: standard deviation; var.: variety.
[a] National monitoring programme.
[b] Value refers to median.

result in bacterial growth which can contribute to the increasing accumulation of high nitrite levels (Chung et al., 2004).

In some studies, the contribution of nitrite is disregarded, considering its low level when compared to nitrate. Therefore the results reported for nitrate in those particular studies are in fact the sum of nitrite and nitrate levels (Prasad & Chetty, 2008; Ximenes et al., 2000).

As regards nitrite levels described in some different studies (Table 4), values <4.77 mg kg$^{-1}$ were shown for cabbage. For lettuce, a maximum value of 25 mg kg$^{-1}$ was presented by Menard et al. (2008) and for spinach a maximum of 220 mg kg$^{-1}$ was reported. For spinach, although 92% of the 112 samples showed nitrite levels < LOD, the estimate of the average nitrite concentration reported is 10.2–18.9 mg kg$^{-1}$. Fytianos and Zarogiannis (1999) reported nitrite values in the range 0.8–8.5 mg kg$^{-1}$ in spinach, with a mean value of 4.0 mg kg$^{-1}$. Although these results are not discussed by the authors, such high levels may be due to the microbial reduction of nitrates to nitrites at room temperature or even under refrigeration. In Portugal, in many supermarkets, during daytime, fresh vegetables, such as spinaches, are normally displayed at room temperature. Therefore nitrate conversion to nitrite may occur and nitrite levels higher than those usually reported in the literature may be observed, especially for those species with naturally high nitrate levels. Considering this possibility, the techniques used in nitrate assessment in commercial vegetable samples should account for the different contributions of both nitrate and nitrite.

According to Chung et al. (2003), there was no significant difference in nitrite levels of vegetables harvested between the summer and the winter. During the storage period, the content of nitrites in frozen spinach products, previously cooked in boiling water for 4 min, increased by 27–42% but did not exceed 1 mg $NO_2^-$ kg$^{-1}$ (Jaworska, 2005b).

The nitrate and nitrite contents of the vegetables contribute to the dietary exposure to nitrate and nitrite. This exposure has been assessed in some recent studies and the results are occasionally compared to the acceptable daily intake (ADI) values. An ADI for nitrate of 0–3.7 mg $NO_3^-$ kg$^{-1}$ body weight (bw) has been set by the Joint Expert Committee of the Food and Agriculture (JEFCA) of the United Nations/World Health Organization (WHO) and the European Commission's Scientific Committee on Food (SCF). For nitrite, the JEFCA and the SCF have proposed an ADI of 0–0.07 and 0–0.06 mg $NO_2^-$ kg$^{-1}$ bw, respectively (Santamaria, 2006).

Pennington (1998) discussed several approaches for estimating dietary exposure to nitrate and nitrite and reported some estimates of nitrate and/or nitrite in daily diets from 1973 to 1997. Intake values can differ to a large extent, depending on the method used for the estimation of additive and contaminant intakes from food (Pennington, 1998; Tamme et al., 2006).

Regarding nitrate, the mean total daily intake per person in Europe ranges between 50 and 140 mg and in the USA about 40–100 mg (Tamme et al., 2006). A comparison of the intake of nitrate and nitrite from vegetables in different countries, with a special focus on recent reports, is shown in Table 5.

The total intake calculated from nine vegetables that account for 80% of the total consumption in the north Chinese diet was 423 mg day$^{-1}$ for nitrate and 0.68 mg day$^{-1}$ for nitrite (Zhong et al., 2002). In New Zealand, the mean dietary exposure to nitrate

Table 4
Nitrite contents (mg kg$^{-1}$) in fresh vegetables (cabbage, lettuce, parsley, spinach and turnip) described in some recent studies.

| Vegetable | Year reported | Author | Country | Nitrite (mg kg$^{-1}$) | | | Number of samples | Notes |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | Minimum | Maximum | | |
| Cabbage | 2002 | Zhong et al. (2002) | China | 0.472 | <LOD | 4.77 | 23 | North China (1998–1999) |
| | 2003 | Chung et al. (2003) | Korea | 0.4 | <LOD | 3.6 | 15 | Winter (November–March), 1998 |
| | | | | 0.3 | <LOD | 2.2 | 25 | Summer (April–October), 1998 |
| | 2006 | Sušin et al. (2006) | Slovenia | 0.2 | LOQ | 0.4 | 52 | (1996–2002)[a]; LOQ = 0.16 mg kg$^{-1}$; 79/151 < LOQ |
| | 2007 | Thomson et al. (2007) | New Zealand | <LOD | | | 8 | November–December, 2003; 2 urban regions LOD = 3.33 mg kg$^{-1}$ |
| | 2008 | Menard et al. (2008) | France | <LOD | | | 7 | Green cabbage (2000–2006)[a] |
| Lettuce | 2000 | Zhou et al. (2000) | China | 0.21 | 0.01 | 0.45 | 6 | Beijing City (1979–1981) |
| | 2003 | Chung et al. (2003) | Korea | 0.6 | <LOD | 2.9 | 15 | Winter (November–March), 1998 |
| | | | | 0.7 | <LOD | 4.6 | 25 | Summer (April–October), 1998 |
| | 2006 | Sušin et al. (2006) | Slovenia | 0.3 | LOQ | 1.4 | 151 | (1996–2002)[a]; LOQ = 0.16 mg kg$^{-1}$; 47/52 samples <LOQ |
| | 2007 | Thomson et al. (2007) | New Zealand | <LOD | | | 18 | November–December, 2003; 2 urban regions LOD = 3.33 mg kg$^{-1}$ |
| | 2008 | Menard et al. (2008) | France | 0.10–6.25[b] | | 25 | 259 | (2000–2006)[a]; 99.6% of the samples <LOD |
| Parsley | 2008 | Menard et al. (2008) | France | <LOD | | | 7 | (2000–2006)[a] |
| Spinach | 1999 | Fytianos and Zarogiannis (1999) | Greece | 4.0 | 0.8 | 8.5 | 12 | |
| | 2000 | Zhou et al. (2000) | China | 0.27 | <LOD | 0.73 | 6 | Beijing City (1979–1981) |
| | 2003 | Chung et al. (2003) | Korea | 0.5 | <LOD | 1.8 | 15 | Winter (November–March), 1998 |
| | | | | 1.2 | <LOD | 5.1 | 25 | Summer (April–October), 1998 |
| | 2005 | Jaworska (2005a) | Poland | 0.34 | | | | SD = 0.16; New Zealand spinach |
| | 2007 | Thomson et al. (2007) | New Zealand | <LOD | | | 8 | November–December, 2003; 2 urban regions LOD = 3.33 mg kg$^{-1}$ |
| | 2008 | Menard et al. (2008) | France | 10.17–18.88[b] | | 220 | 112 | (2000–2006)[a]; 92% of the samples <LOD |
| Turnip | 2000 | Zhou et al. (2000) | China | <LOD | | | 1 | Beijing City (1979–1981) |
| | 2008 | Menard et al. (2008) | France | <LOD | | | 13 | (2000–2006)[a] |

LOD: limit of detection; SD: standard deviation; var.: variety.
[a] National monitoring programme.
[b] Lower and upper bounds for the estimate of the average concentration.

Table 5
Comparison of the intakes of nitrate and nitrite from vegetables in different countries (mg person$^{-1}$ day$^{-1}$) using a basis of 60 kg body weight.

| Country | Year report | Author | Vegetable consumption (g day$^{-1}$) | Nitrate intake (mg day$^{-1}$) | Nitrite intake (mg day$^{-1}$) | % ADI nitrate | % ADI nitrite | Notes |
|---|---|---|---|---|---|---|---|---|
| China | 2002 | Zhong et al. (2002) | 444 | 422.8 | 0.68 | 219 | | North China; nine vegetables that account for 80% of the total consumption in the north Chinese diet |
| Denmark | 1999 | Petersen and Stoltze (1999) | 142 | 38.9 | 0.091 | | | |
| England | 1999 | Ysart et al. (1999) | 322 | 109 | 2.2 | | | |
| Egypt | 1998 | Saleh, Brunn, Paetzold, and Hussein (1998) | 159 | 296 | – | 116 (F) 140 (M) | | $(NO_2^- + NO_3^-)$; total diet study |
| France | 2008 | Menard et al. (2008) | 67.1 | | | 27 | <0.05–33.3 | Adults (>15 year-old), *n* = 1474; excluding potatoes |
| | | | 111 | 90 | 1.2–2.4 | 24.3 | <0.05–16.7 | Adults (>15 year-old), *n* = 1474; excluding potatoes |
| Korea | 2003 | Chung et al. (2003) | 104 | 182 | 0.12 | | | Chinese cabbages only |
| Italy | 2003 | De Martin and Restani (2003) | 34.1 | 65.8 | – | 29.8 | | Adults; includes the five most consumed vegetables, supposed to provide most of the nitrate intake in the typical Italian diet |
| | | | 8.5 | 14.5 | | 19.6 | | Children (4–6 years, bw = 20 kg); includes the 5 most consumed vegetables, supposed to provide most of the nitrate intake in the typical adult Italian diet |
| Estonia | 2006 | Tamme et al. (2006) | 376 | 58 | | 26 | | Adults, Consumption Survey National Board of Statistics |
| | | | 226 | 30 | | 40 | | Children (4–6 year-old), two kindergarten menu consumption survey |
| | | | 198 | 26 | | 52 | | Children (1–3 year-old), recommended infant menu |
| New Zealand | 2007 | Thomson et al. (2007) | 231 | 42 | 0.48 | 16 | 13 | Adults; food and water combined. |
| New Zealand | 2004 | | | 31.2 | 0.54 | | | Total diet, additives plus vegetables, 2004 |

from food and water combined was estimated to be 16% of the ADI, with a median exposure level of 9% of the ADI. Approximately 1% of exposure scenarios resulted in daily dietary exposures above the ADI for nitrate (Thomson et al., 2007). The most significant contributors were potatoes (32%) and lettuce (29%). The mean dietary exposure to exogenous nitrite was about 13% of the ADI, excluding endogenous conversion of nitrate. If a contribution from the conversion of dietary nitrate to nitrite was considered, the ADI was exceeded 10% of the time for an average conversion rate (5%) and 50% of the time for individuals with high conversion rate (20%) (Thomson et al., 2007).

Nitrate dietary exposure in France averaged 40% of the ADI for adults and 51–54% for children (Menard et al., 2008). The major contributors were, for adults and children, respectively, vegetables (24% and 27% of ADI), potatoes (5% and 11% of ADI) and water (5% and 5% of the ADI). Nitrite dietary exposure averaged 33–67% of the ADI for adults and 67–133% for children. This was the first French estimate of the dietary exposure to nitrate and nitrite based on concentrations analysed in foods (13,657 analyses in foods and 6870 analyses in water) (Menard et al., 2008).

The knowledge of the nitrate and nitrite metabolisms in humans and their toxicological effects must be improved to better characterise the risk of dietary exposure to nitrate and nitrite present in food (Menard et al., 2008). Case-control studies examining dietary intake of nitrate and the risk of gastric cancer have consistently found a negative association (Kelley & Duggan, 2003). In such studies, vegetable intake has been consistently related to a decreased risk of gastric cancer. Nitrate intake was probably an index of vegetable intake, which can explain the negative association. Recent case-control studies have all reported a weak, statistically non-significant increased risk of gastric cancer for high vs. low nitrite intake. Nitrite intake may be related to the consumption of preserved meat, typically high-salt foods, which can also contribute to an increased risk of gastric cancer. It is interesting to note that a diet high in nitrite does not appear to confer an increased risk if that diet is also high in antioxidants from fruits and vegetables (Kelley & Duggan, 2003).

## 4. Conclusions

Growing concern over nitrate toxicity has produced a number of studies on nitrate and nitrite contents of fresh vegetable samples. Nitrite and nitrate levels of 34 vegetable samples, from an intensive horticultural area in north Portugal, were determined and their levels compared to those reported in recent literature. No maximum limits established for nitrate were exceeded and the results are in the range of others reported in different countries. According to actual knowledge, the ingestion of these vegetables is supposed to be beneficial for the population despite their nitrate and nitrite contents.

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