Food Additives and Contaminants

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: https://www.tandfonline.com/loi/tfac20

Monitoring programme on nitrates in vegetables and vegetable-based baby foods marketed in the Region of Valencia, Spain: levels and estimated daily intake

O. Pardo-Marín , V. Yusà-Pelechà , P. Villalba-Martín & J.A. Perez-Dasí

Food Additives and Contaminants
Vol. 27, No. 4, April 2010, 478–486

Taylor & Francis
Taylor & Francis Group

Monitoring programme on nitrates in vegetables and vegetable-based baby foods marketed in the Region of Valencia, Spain: levels and estimated daily intake

O. Pardo-Marín*, V. Yusà-Pelechà, P. Villalba-Martín and J.A. Perez-Dasí

Conselleria de Sanitat, Food Safety Area, Public Health Laboratory of Valencia, Public Health Research Center (CSISP), Avda. de Cataluña, 21, 46020 Valencia, Spain

(*Received 29 August 2009; final version received 26 October 2009*)

This study was carried out to determine the current levels of nitrates in vegetables and vegetable-based baby foods (a total of 1150 samples) marketed in the Region of Valencia, Spain, over the period 2000–2008, and to estimate the toxicological risk associated with their intake. Average (median) levels of nitrate in lettuce, iceberg-type lettuce and spinach (1156, 798 and 1410 mg kg^{-1} w/w, respectively) were lower than the maximum limits established by European Union legislation. Thirteen fresh spinach samples exceeded the regulatory limits. Median nitrate values in other vegetables for which a maximum limit has not been fixed by the European Commission were 196, 203, 1597, 96, 4474 and 2572 mg kg^{-1} w/w (for potato, carrot, chard, artichoke, rucola and lamb's lettuce, respectively). The estimated nitrate daily intakes through vegetables consumption for adult, extreme consumers and children were found to be about 29%, 79.8% and 15.1%, respectively, of the acceptable daily intake (3.7 mg kg^{-1}). The levels (median = 60.4 mg kg^{-1} w/w) found in vegetable-based baby foods were, in all cases, lower the maximum level proposed by European Union legislation. The estimated nitrate daily intake through baby foods for infants between 0–1 and 1–2 years of age were 13% and 18%, respectively, of the acceptable daily intake.

Keywords: exposure; high-performance liquid chromatography (HPLC); risk assessment; quality assurance; nitrate; baby food; vegetables

Introduction

Vegetables play an important role in human nutrition since they are an outstanding source of vitamins, minerals and biologically active compounds (Favell 1998). The health benefits of a diet rich in vegetables and fruits have been recognized for some time. The World Health Organization (WHO) recommends that one consumes at least 400 g, or five portions of fruits and vegetables (excluding tubers), a day (WHO 2003). However, these plant foods can also be a source of unfavourable contaminants, e.g. nitrates, heavy metals and residues of pesticides.

Several sources are responsible for the dietary intake of nitrates, of which vegetables and drinking water are quantitatively the most important (De Martin and Restani 2003). Nitrates and nitrites are also used as antimicrobial and flavouring/colouring agents in meat and fish products. About 90% of the total nitrate concentration in a European normal diet is believed to be a direct result of vegetable intake (Hambridge 2003). The presence of nitrates in vegetables is one of the consequences of the mechanism by which plants absorb the nitrogen element, in the form of NO_3^-, from fertilizers or organic materials (Schuddeboom 1993), which are essential to the process of protein synthesis. Their concentration depends on the biological properties of the plant culture, lightning conditions, type of soil, temperature, humidity, frequency of plants in the field, plant maturity, vegetation period, harvesting time, size of vegetable unit, storage time and source of nitrogen (Fytianos and Zagoriannis 1999; Muramoto 1999). It has been discovered that green leafy vegetables contain the highest levels of nitrates (Wolff and Wasserman 1972). 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, cauliflowers, French beans, peas and potatoes seldom accumulate nitrates. The nitrate content of vegetables may range from 1 to 10,000 mg kg^{-1} (Ximenes et al. 2000). The limit concentrations for nitrates have been established in the European Union for spinach, lettuce and baby foods (European Commission 2006a). The maximum limit (ML) prescribed for lettuce (2000–4000 mg kg^{-1} of fresh sample) depend on the time of yield harvest and

*Corresponding author. Email: polgueta@gmail.com

ISSN 0265–203X print/ISSN 1464–5122 online

DOI: 10.1080/19440040903439804
http://www.informaworld.com

the type of growing (in the open or under protected conditions), and for spinach (2000–3000 mg kg^{-1} of fresh sample) on the time of yield harvest and the type of processing (canned or frozen spinach).

The nitrate ion has a low level of acute toxicity, but if transformed into nitrite, it may constitute a health problem. Some researches suggested that the vegetables with high nitrate in the diet could put a human at risk of gastrointestinal cancer and methemoglobinaemia (Bartsch et al. 1988). It has been estimated that 5–8% of the nitrate from the diet may be reduced to nitrite by microflora in the oral cavity (Mensinga et al. 2003). The elevation of gastric pH >5.5 leads to bacterial growth followed by rapid conversion of nitrate to nitrite. Nitrite is a precursor in the formation of nitrosamines (Tannenbaum and Correa 1985). Although carcinogenicity of N-nitrosoamines in humans cannot be tested, epidemiological studies have suggested a possible link to the incidence of various cancers in humans (Pegg and Shahidi 2000). Vegetables are an important part of most babies' diets (Huarte-Mendicoa et al. 1997). Consumption of vegetables containing high level of nitrates and incorrect storage of home-made vegetable purees has been found to be potential causes of infant methemoglobinaemia (Sanchez-Echaniz and Benito-Fernández 2001). Processed infant foods may also contain increased levels of nitrites (Hill 1996). Young babies with low stomach acidity may suffer from infantile methemoglobinaemia due to excessive nitrates in their diet, where nitrite is substituted for oxygen in haemoglobin and death may occur (Swann 1975; Ezeagu 1996).

Therefore, there is great concern about nitrate content in the daily diet, especially in vegetables. In recent decades, considerable researches have been done to minimize the nitrate accumulation in vegetables, mainly in plant nutrient management, horticultural technology, and breeding. Interest in the dietary intakes of nitrates and nitrites has arisen from the concern about their possible adverse effect on health. An acceptable daily intake (ADI) of 0–3.7 mg kg^{-1}, equivalent to 222 mg nitrate day^{-1} for a 60 kg adult, was established by the former Scientific Committee for Food (European Union Scientific Committee for Food (SCF) 1995) and was reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002.

The Food Safety Plan of Valencia Public Health Department in Spain contains a specific monitoring programme focused on nitrates in vegetables and vegetable-based baby foods. The aim of the present study is to present the results of the monitoring of nitrate content in vegetables and vegetable-based baby foods marketed in Comunitat Valenciana over the period 2000–2008 to compare them with the results of monitoring being performed in countries of the European Union and around the world and to estimate the toxicological risk associated with their intake.

Materials and methods

Samples and sampling

Samples of vegetables and vegetable-based baby foods were randomly acquired in local markets, large supermarkets and grocery stores of the Valencia region and stored at 4°C until analysis. The vegetable species included in the study are lettuce (*Lactuca sativa*), iceberg-type lettuce (*Lactuca sativa* var. *capitata*), spinach (*Spinacia oleracea*), potato (*Solanum tuberosum*), carrot (*Daucus carota*), chard (*Beta vulgaris* var. *cicla*), artichoke (*Cynara scolymus*), rucola (*Eruca sativa*), and lamb's lettuce (*Vallerianella locusta*). These species were chosen because of their high consumption in the Valencian diet.

Nitrate concentrations in vegetables vary according to season and condition of cultivation, as well as to the storage conditions. Therefore, the following food commodities were collected: fresh spinach, preserved, deep-frozen or frozen spinach; fresh lettuce (harvested between 1 October and 31 March and harvested between 1 April and 30 September) or iceberg-type lettuce grown under cover and in the open; and vegetable-based products for infants and young children.

All samples (1083 vegetables and 67 baby foods samples) were collected during 2000–2008 by food inspectors/authorities within the frames of state official surveillance and the monitoring programme and analysed in an accredited public laboratory, the Public Health Laboratory of Valencia.

Reagent and standard solutions

Nitrate calibration standard solutions were prepared from certified commercial standard solutions (Merck, Darmstadt, Germany) by dilution in water and stored in capped amber vials at 4°C. Concentrations of the calibration standards ranged between 0.5 and 100 μg ml^{-1}. Water was Milli-Q water from a Millipore purification system (Millipore, Bedford, MA, USA). Borax was supplied by Panreac (Barcelona, Spain). Carrez I and II were purchased from Scharlau (Barcelona, Spain). Methanol, monopotassium phosphate and disodium phosphate were of high analytical grade (Merck). Nitrate reference test materials were provided by CSL (Central Science Laboratory, York, UK).

Sample treatment

Before testing, any inedible parts were removed. The whole samples were quartered, ground and homogenized (Ultra-Turrax TR-50, IKA, Staufen, Germany). An analytical aliquot of approximately 5 g was weighed. The subsample was transfer into a 200 ml volumetric flask and about 150 ml of water and 5 ml of borax solution were added. The whole content was shaken, and after 15 min in warm water, 2 ml of Carrez I and 2 ml of Carrez II were added. The mixture was then brought to volume and filtered into a 250 ml Erlenmeyer flask through a 0.45-µm pleated filter.

An SPE column (C18/1 g, Sep-Pak®, Waters Corporation, Milford, MA, USA) was activated with 5 ml methanol and 5 ml Milli Q water. A total of 4 ml of the filtered sample were loaded onto the column to saturate it and the eluate was discarded; another 4 ml of the sample were then passed through. The final extract was collected and filtered through 0.45 µm before the liquid chromatographic (LC) analysis.

Instrumental

The liquid chromatograph was an isocratic system equipped with an ultraviolet (UV) diode array detector (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separations were performed at 30°C on a Waters IC-PAK HC anion exchanger column (4.6 × 150 mm) using a 4.6 × 50 guard column of the same phase (Waters Corporation, Milford, MA, USA). The mobile phase was phosphate 5 mM (pH 6.5) at a flow rate of 1.2 ml min^{-1}. The injection volume was 20 µl; the total run time of the chromatograms was 20 min. The UV detector operated at 214 nm and a 200–400 nm spectrum was registered.

Method validation and analytical quality assurance (AQA)

The analytical method was validated. The main performance characteristics of the method were: limits of detection (LOD) and quantitation (LOQ) of 7 and 20 mg kg^{-1}, respectively; recoveries from 83% to 106%; and precision between 5% and 12%, estimated as the relative standard deviation (less than the recommended values derived from Horwitz equation). The performance criteria fulfil the specific criteria for methods of analysis used in the monitoring of nitrate levels established in European legislation (European Commission 2006b). The linear model was statistically validated taking into account that residual values were randomly distributed about the regression line, the *p*-value of the *F*-test statistic was <0.05, and the coefficient of determination (R^2) was >0.999 (Eurachem Guide 1998).

The laboratory was accredited by the Spanish national body for accreditation (ENAC), and it operated under the quality assurance system established by ISO/IEC 17025 (International Organization for Standardization/International Electrotechnical Commission 2005). All test batches were analysed under quality-assurance protocols, including duplicate samples, reagent blanks, spiked samples, and certified reference materials from FAPAS (Central Science Laboratory (CSL), Sand Hutton, York, UK). Six lettuce (FAPAS T1529, T1531, T1537, T1543, T1546 and T1555) and four spinach (FAPAS T1532, T1548, T1551 and T1557) reference materials were analysed during the period 2003–2008. The results of analysis (*z*-scores ranged from −1.1 to 1.5) were in good agreement with reference values. The laboratory participates regularly in laboratory proficiency testing provided by the CSL, with satisfactory *z*-scores.

Dietary exposure estimates

The daily intake of nitrates through vegetables was calculated by multiplying the respective average concentration (median) by the weight of vegetables consumed by an average adult from Spain (17 years and over; 68.5 kg body weight) per day. Also, exposures for children (7–12 years; average weight, 34.5 kg) and adult extreme fish consumers were estimated:

$$\text{Daily intake (mg week}^{-1}) = \text{occurrence (mg kg}^{-1}) \times \text{consumption (kg person}^{-1}\,\text{day}^{-1})/\text{body weight (kg)}.$$

The daily intake of nitrates through vegetable-based baby foods was calculated by multiplying the respective average concentration (median) by the weight of vegetable-based baby foods consumed by an average baby from Spain (0–1 years, 7.4 kg body weight; 1–2 years, 11.7 kg body weight) per day.

In this work food consumption data from the Spanish Food Safety Authority (Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) 2006) were used in the calculation of average intake for the whole population (children 7–12 years, adults and extreme consumers). Intake of nitrates by infants (0–1 and 1–2 years) were estimated using the consumption data from the Spanish Nuclear Safety Council (Consejo de Seguridad Nuclear (CSN) 2002).

For calculations, when a nitrate concentration was under the LOQ, the value was assumed to be the respective limit, which was 20 mg kg^{-1}.

Results and discussion

Concentration of nitrates in vegetables

Nitrate concentrations found, expressed in mg kg^{-1} of wet weight (w/w), in the different vegetable species monitored are summarized in Table 1.

Cultivar and harvest date can affect the nitrate levels of selected vegetables (Amr and Hadidi 2001).

Table 1. Nitrate contents of vegetables marketed in the region of Valencia during the period 2000–2008.

Vegetable species	Number of samples	Minimum nitrate content ($mg\,kg^{-1}$)	Maximum nitrate content ($mg\,kg^{-1}$)	Median content ($mg\,kg^{-1}$)[a]
Lettuce (*Lactuca sativa*)	299	<20	1623	1156
Harvested 1 October–31 March				
Grown under cover	82	447	1982	1467
Grown in the open air	50	126	1632	1208
Harvested 1 April–30 September				
Grown under cover	88	<20	1423	846
Grown in the open air	79	<20	1367	808
Iceberg-type lettuce (*Lactuca sativa* var. capitata)	81	195	1878	798
Grown under cover	45	195	1878	847
Grown in the open air	36	306	1154	723
Spinach (*Spinacia oleracea*)	206	<20	5102	1410
Fresh spinach harvested 1 October–31 March	102	267	5102	1755
Fresh spinach harvested 1 April–30 September	72	142	3845	1269
Preserved, deep-frozen or frozen spinach	32	<20	2067	905
Potato (*Solanum tuberosum*)	110	<20	471	196
Carrot (*Daucus carota*)	112	<20	833	203
Chard (*Beta vulgaris* var. *cicla*)	174	<20	4677	1597
Artichoke (*Cynara scolymus*)	44	<20	181	96
Rucola (*Eruca sativa*)	32	2320	8365	4474
Lamb's lettuce (*Vallerianella locusta*)	25	927	4109	2572

Note: [a]Median concentrations listed were determined using all samples.

For this reason, European legislation (European Commission 2006a) fixes different maximum nitrate levels in certain vegetables such as lettuce and spinach depending on the cultivar and the season.

Table 1 shows that spinach has the highest nitrate content (median = 1410 $mg\,kg^{-1}$) compared with the other vegetable species (lettuce and iceberg-type lettuce) whose maximum limits are regulated. This finding correlated well with the levels found in other Mediterranean countries as France or Italy (Malmauret et al. 2002; De Martin and Restani 2003; Menard et al. 2008) and are lower than those reported by Tamme et al. (2006) or Hsu et al. (2009) from Estonia and Australia, respectively. The levels found were, in general, below the maximum limits established by the European Commission (2006a). However, 13 fresh spinach samples exceeded the regulatory limits (Table 2): ten were summer samples and three winter samples. The nitrate concentration in some crops of different varieties during summer and winter seasons were screened by Escobar-Gutierrez et al. (2002): the nitrate concentration showed great variability between cultivars and also varieties within one cultivar, exceeding the limit of concentrations was more frequent in the summer season than in winter. As can be seen in Table 1, spinach harvested during autumn–winter (median = 1755 $mg\,kg^{-1}$) has a higher content of nitrate when compared with those harvested in spring–summer (median = 1269 $mg\,kg^{-1}$). These differences can be explained by both higher irradiance in summer, which tends to reduce nitrate, and also to higher growth rates, which coincide with periods of high irradiance and warmer temperatures (Kannan and Economakis 1992). However, according to Kmiecik et al. (2004), processed spinach (frozen, deep-frozen or preserved) contains the lowest levels of nitrate due to the reduction of the content during the blanching process.

For lettuce and iceberg-type lettuce, the nitrate contents found (median = 1156 and 798 $mg\,kg^{-1}$, respectively) are similar to those reported by Malmauret et al. (2002) and Merino et al. (2006), respectively, in studies carried out in France. Iceberg-type lettuce levels are considerably higher than those reported by Hsu et al. (2009) in Australia, and lettuce levels are lower than those reported by Tamme et al. (2006) in Estonia. As can be seen in Table 1, fresh lettuce grown under cover (with potentially reduced light intensity) contain higher levels of nitrate compared with those grown in the open air (both collected in winter and summer seasons). The nitrate values found in lettuce and iceberg-type lettuce were, in all cases, below the maximum limits established by the European Commission (2006a).

Nitrate content in other vegetables (potato, carrot, chard, artichoke, rucola and lamb's lettuce) for which a maximum limit has not been fixed by the European Commission were investigated. The species with the highest content of nitrates were rucola

Table 2. Nitrate contents in fresh spinach samples (marketed in the region of Valencia) exceeding the regulatory limits during the period 2000–2008.

Harvesting date	Nitrate content ($mg\,kg^{-1}$)	Regulatory limit ($mg\,kg^{-1}$)[a]
June 2001	2706	2500
October 2001	2761	2500
June 2002	2976	2500
June 2002	3485	2500
October 2002	4491	2500
November 2002	3215	3000
October 2003	3505	2500
October 2003	2996	2500
December 2003	5102	3000
October 2004	3117	2500
October 2005	2832	2500
October 2005	2680	2500
March 2007	3173	3000

Note: [a]Established in Commission Regulations (EC) No. 466/2001 (European Commission 2001) and No. 1881/2006 (European Commission 2006a).

(median = 4474 $mg\,kg^{-1}$), lamb's lettuce (median = 2572 $mg\,kg^{-1}$), and chard (median = 1597 $mg\,kg^{-1}$). Rucola levels are higher than those reported in Italy by De Martin and Restani (2003). The use of rucola and lamb's lettuce seems to be increasing in several European diets, therefore it can mean a large contribution to the daily intake of nitrate. Elevated consumption of rucola, more than 47 g, at the median nitrate concentration means that one will exceed the ADI without taking into account any other source of nitrate exposure (European Food Safety Authority (EFSA) 2008). The lowest median values of nitrates were detected in artichoke, potato and carrot. The content of nitrates in artichoke (median = 96 $mg\,kg^{-1}$) is higher than levels found in France (Menard et al. 2008). The median nitrate content of potato (median = 196 $mg\,kg^{-1}$) is also comparable with those obtained in France (Menard et al. 2008), China (Zhong et al. 2002), and Slovenia (Susin et al. 2006) with median values of 191.8, 164, and 158 $mg\,kg^{-1}$, respectively. Ierna (2009) has recently investigated the influence of variety and harvest time in potato nitrate content, concluding that tuber nitrate contents vary between potato varieties and that nitrate contents decrease in winter–spring crops, whereas they increase in summer–autumn crops. The nitrate level found in carrot was 203 $mg\,kg^{-1}$ and is similar to that found in Slovenia (Susin et al. 2006).

The nitrate levels in the different vegetables show similar profiles (Figure 1) to those reported by Tamme et al. (2006), Menard et al. (2008) or Susin et al. (2006) in Estonia, France and Slovenia, respectively. Leafy vegetables accumulate high amounts of nitrates: concentrations reaching up to 6000 $mg\,kg^{-1}$ (Tamme et al. 2006). On the other hand, roots and tubers contain generally low nitrate levels.

Concentration of nitrates in vegetable-based baby foods

Sixty-seven samples of vegetable-based baby foods were analysed and the levels found were, in all cases, lower the maximum level proposed by European legislation (European Commission 2006a). A nitrate content close to the European Union limit concentration of 200 $mg\,kg^{-1}$ was detected in one sample.

The mean nitrate content in vegetable-based baby foods (taking into account the samples with concentrations above the minimum reporting levels) was 60.4 $mg\,kg^{-1}$ with a standard deviation of 38.6 $mg\,kg^{-1}$. This level is lower than those in a Spain (Hardisson et al. 1996) and Estonia (Tamme et al. 2006), where mean levels of 92 and 88 $mg\,kg^{-1}$ were reported. Tamme et al. (2006) detected the highest levels in baby foods containing carrot and pumpkin (62–148 and 124–162 $mg\,kg^{-1}$, respectively). Similar results have been reported in a Spanish study (Hardisson et al. 1996), where nitrate concentration exceeding the regulatory limit was found in baby foods in which the main ingredient was carrot.

Evaluation of dietary exposure

Human exposure to nitrate is mainly exogenous through the consumption of vegetables, and to a lesser extent through water and other foods (nitrate is an approved food additive). It is estimated that approximately 80–95% of daily nitrate intake comes from plant foods, mostly vegetables and a few fruits (Pennington 1998). Consequently, information about dietary intake is necessary in order to evaluate the potential risk for humans. Apart from comparing the concentrations of nitrate with the maximum levels fixed by European legislation (European Commission 2006a), a more significant assessment of potential health hazards for consumers can be obtained by calculating the daily nitrate intake derived from the consumption of vegetables and comparing it with the ADI set by international organizations. It should be stated that the purpose here is not to evaluate the total diet exposure, but rather to study the intake of nitrate from vegetables. Vegetables with the highest consumption values for the total Spanish population (AESAN 2006) have been selected for the estimation of daily intake.

An ADI for nitrate of 3.7 $mg\,kg\ bw^{-1}\,day^{-1}$, equivalent to 222 mg nitrate day^{-1} for a 60-kg adult established by the former Scientific Committee on Food (European Union SCF 1995). Table 3 shows the nitrate daily intake through vegetables based on the

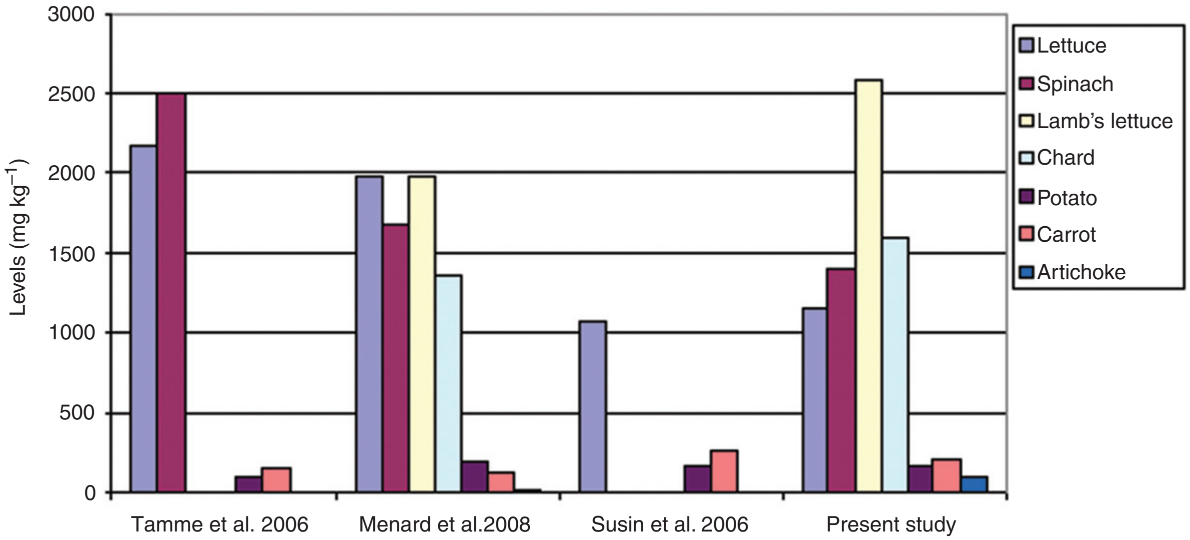

Figure 1. Profile of vegetables nitrate contents (median values) in different studies. Median concentration listed in the present study were determined using all samples.

Table 3. Estimation of the nitrate dietary intake through vegetables consumption based on the median concentrations found in the study.

Commodity	Occurrence ($mg\,kg^{-1}$)	Consumption ($g\,day^{-1}$ per person[a])			Daily intake ($mg\,kg\,bw^{-1}\,day^{-1}$)			Per cent ADI		
		Adults		Children	Adults		Children	Adults		Children
	Median	Mean	High level[b]	Mean	Mean	High level	Mean	Mean	High level	Mean
Lettuce	1156	35.5	76.6	13.9	0.6	1.3	0.2	17.1	36.9	6.7
Potato	196	56.8	109.3	55.0	0.2	0.3	0.2	4.6	8.9	4.5
Carrot	203	9.3	24.9	5.9	0.0	0.1	0.0	0.8	2.1	0.5
Spinach	1410	4.9	24.2	2.7	0.1	0.5	0.1	2.9	14.2	1.6
Chard	1597	5.2	24.2	2.6	0.1	0.6	0.1	3.5	16.1	1.7
Artichoke	96	2.8	15.3	1.0	0.0	0.0	0.0	0.1	0.6	0.0
Total					1.0	2.8	0.5	29.0	78.9	15.1

Notes: [a]Average adult (17 years and over) weight: 68.5 kg; average children (7–12 years) weight: 34.5 kg.
[b]Increasing by 1 SD (standard deviation) the average consumption of each vegetable item.
ADI, acceptable daily intake.

concentrations found in this study, calculated by taking into account the consumption data per adult and per child for the total Spanish population (AESAN 2006). The extreme exposures (high level of vegetables consumption) are also shown in Table 3.

The nitrate daily intake through vegetables was 69 mg per person day^{-1}, which corresponds to 29% of the ADI. This intake is similar than those reported by other countries, as can be seen in Table 4. The main contribution to the daily intake comes from lettuce, with medium levels of nitrates, and is approximately six times higher than the contribution from spinach. The use of rucola and lamb's lettuce seems to be increasing in Spanish salads, so a high consumption of these vegetable spices can contribute to an increase in the daily nitrate intake. Consumption of rucola of more than 47 g day^{-1} leads to an excursion above the ADI without taking into account any other source of nitrate exposure (EFSA 2008). On the other hand, potatoes often containing low levels of nitrates, but eaten more frequently, have an important influence in total dietary intake, similar for adults and children, and it means an important contribution for the daily nitrate intake of children, since they are an important part of their diet (approximately 4.5%). The contribution of chard to the daily intake of nitrate is slightly higher than that reported for spinach. The nitrate intakes from artichoke and carrot consumption (0.1% and 0.8% of the ADI, respectively) are the lowest of the commodities studied.

For children, the mean daily nitrate intake from vegetables is 15.1% of the ADI. This value is directly

Table 4. Estimated mean nitrate daily intake through vegetables and per cent acceptable daily intake (ADI) in different studies.

Country	Year of study	Estimated daily intake (mg kg bw^{-1} day^{-1})	Per cent ADI	References
Netherlands	1997	47.7	21.6	Van Vliet et al. (1997)
Italy	2003	65.8	29.8	De Martin and Restani (2003)
United Kingdom	2003	53	23.9	Clarke et al. (2003)
New Zealand	2004	51	23	Thomson (2004)
Estonia	2006	58	26.3	Tamme et al. (2006)
France	2008	52.9	24	Menard et al. (2008)
Spain	2009	54.8	27.1	Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) (2009)
Spain	2009	69	29	Present study

Table 5. Estimation of the nitrate dietary intake through vegetable-based baby foods consumption based on the mean concentrations found in the study.

Age (years)	Occurrence (mg kg^{-1})	Average body weight (kg)	Consumption (g day^{-1} per infant)	Daily intake (mg kg bw^{-1} day^{-1})	Per cent ADI
0–1	60.4	7.4	35.5	0.5	13
1–2	60.4	11.7	56.8	0.7	18

Note: ADI, acceptable daily intake.

related to the low consumption of green salad by children. The estimation of the dietary exposure for individuals who eat huge amounts of vegetables shows that these extreme vegetable consumers have a nitrate daily intake of 78.9% of the ADI. This value is higher than that reported by Clarke et al. (2003) in vegetarian diets.

The daily nitrate intake through vegetable-based baby foods based on the concentrations found in this study, calculated by taking into account the consumption data of Spanish infants (CSN 2002), is shown in Table 5. The estimated daily nitrate intake through vegetable-based baby foods corresponds to 13% and 18% of the ADI for infants from 0 to 1 year and from 1 to 2 years, respectively. This intake is lower than those reported by Tamme et al. (2006) and the estimated value is adequate in order to protect against infant methemoglobinaemia.

Conclusions

Taking into account the vegetable species considered, the data obtained in this study do not show evidence of risk for average consumers. Nitrate values were, in general, lower than the maximum limits established by European Union legislation. Thirteen fresh spinach samples, ten summer samples and three winter samples exceeded the regulatory limits. In the case of vegetable-based baby foods, the levels found were, in all cases, lower than the maximum level proposed by European Union legislation.

This study also reports the dietary intakes of nitrates through vegetable consumption in adults living in the Region of Valencia on the basis of food consumption data at the national level. It turns out that the contribution of vegetables to the intake of nitrate is extensively below the ADI values indicated by international regulation bodies. The estimated daily intake accounts for 29% of the ADI, which is similar to those found in other countries. The estimated daily intake for extreme vegetable consumers is 78.9% of the ADI, which is higher than that reported in studies of vegetarian diets. Although the main human exposure to nitrate is through the consumption of vegetables, it should be highlight that for the total nitrate daily intake the contribution of water and meat products must be taken into account.

Acknowledgements

The authors are grateful to all inspectors from the Public Health Department of Valencia Regional Government who participated in the monitoring programme. They also thank the technicians of the Public Health Laboratory of Valencia, Spain.

References

Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). 2006. Modelo de dieta española para la determinación de la exposición del consumidor a sustancias químicas. Madrid (Spain): Ministerio de Sanidad y Consumo. Available from: http://www.aesan.msps.es/aesan/

Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). 2009. Resumen de los datos de control de nitratos en alimentos. Año 2008. Madrid (Spain): Ministerio de Sanidad y Consumo. Available from: http://www.aesan.msps.es/aesan/

Amr A, Hadidi N. 2001. Effect of cultivar and harvest date on nitrate (NO_3) and nitrite (NO_2) content of selected vegetables grown under open field and greenhouse conditions in Jordan. J Food Composit Anal. 14:59–67.

Bartsch H, Ohshima H, Pignatelli B. 1988. Inhibitors of endogenous nitrosation: mechanisms and implications in human cancer prevention. Mutat Res. 202:307–324.

Clarke DB, Barnes KA, Castle L, Martin R, Wilson LA, Baxter MJ, Price KR, DuPont Ms. 2003. Levels of phytoestrgens, inorganic trace-elements, natural toxicants and nitrate in vegetarian duplicate diets. Food Chem. 81:287–300.

Consejo de Seguridad Nuclear (CSN). 2002. Estudio sobre dietas y hábitos alimentarios en la población española. Madrid (Spain): CSN.

De Martin S, Restani P. 2003. Determination of nitrates by novel ion chromatographic method: occurrence in leaky vegetables (organic and conventional) and exposure assessment for italian consumers. Food Addit Contam. 20:787–792.

Escobar-Gutierrez AJ, Burns IG, Lee A, Edmondson RN. 2002. Screening lettuce cultivars for low nitrate content during summer and winter production. J Horticult Sci Biotechnol. 7(2):232–237.

Eurachem Guide. 1998. The fitness purpose of analytical methods. A laboratory guide to method validation and related topics. Teddington (UK): LGC.

European Commission. 2001. Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Comm. L77:1–13.

European Commission. 2006a. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Comm. L364:5–24.

European Commission. 2006b. Commission Regulation (EC) No. 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrates in certain foodstuffs. Off J Eur Comm. L364:25–31.

European Food Safety Authority (EFSA). 2008. Nitrate in vegetables. Scientific Opinion of the Panel on Contaminants in the Food Chain. EFSA J. 689:1–79.

European Union Scientific Committee for Food (SCF). 1995. Opinion on nitrate and nitrite. Expressed on 22 September 1995. Brussels (Belgium): European Commission DG III. Annex 4 to document III/56/95, CS/CNTM/NO3/20-FINAL.

Ezeagu IE. 1996. Nitrate and nitrite contents in ogi and the changes occurring during storage. Food Chem. 56: 77–79.

Favell DJ. 1998. A comparison of the vitamin C content of fresh and frozen vegetables. Food Chem. 62:59–64.

Fytianos K, Zagoriannis P. 1999. Nitrate and nitrite accumulation in fresh vegetables from Greece. Bull Environ Contam Toxicol. 62:187–192.

Hambridge T. 2003. Nitrate and nitrite: intake assessment. Geneva (Switzerland): World Health Organization, Geneva. WHO Food Additives Series. 50:1053–1071.

Hardisson A, González-Padrón A, Frías I, Reguera JI. 1996. The evaluation of the content of nitrates and nitrites in food products for infants. J Food Composit Anal. 9:13–17.

Hill M. 1996. Nitrates and nitrites in food and water. Cambridge: Woodhead.

Hsu J, Arcot J, Lee NA. 2009. Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. Food Chem. 115:334–339.

Huarte-Mendicoa JC, Astiasaran I, Bello J. 1997. Nitrate and nitrite levels in frozen broccoli. Effect of freezing and cooking. Food Chem. 58:39–42.

Ierna A. Influence of harvested date on nitrate contents of three potato varieties for of-season production. J Food Composit Anal. 22:551–555.

International Organization for Standardization/International Electrotechnical Commission. 2005. ISO/IEC 17025:2005: General requirements for competence of testing and calibration laboratories. Available from: http://www.iso.org

Joint FAO/WHO Expert Committee on Food Additives (JEFCA). 1996. Nitrate. Toxicological evaluation of certain food additives and contaminants in food. WHO Technical Report Series 913:20–32.

Kannan SS, Economakis CD. 1992. Effect of climatic conditions and time of harvest on growth and tissue nitrate content of lettuce in nutrient film culture. Acta Hort. (ISHS) 323:75–80.

Kmiecik W, Cisiewska Z, Slupski J. 2003. Effects of freezing and storing of frozen products on the content of nitrate, nitrites and oxalates in dill (*Anethurm graveolons* L.). Food Chem. 86:105–111.

Malmauret L, Parent-Massin D, Hardy JL, Verger P. 2002. Contaminants in organic and conventional foodstuffs in France. Food Addit Contam. 19:524–532.

Menard C, Heraud F, Volatier JL, Leblanc JC. 2008. Assessment of dietary exposure of nitrate and nitrite in France. Food Addit Contam. 25:971–988.

Mensinga TT, Speijers GJA, Meulenbelt J. 2003. Health implications of exposure to environmental nitrogenous compounds. Toxicol Rev. 22(1):41–51.

Merino L, Darnerud PO, Edberg U, Aman P, Castillo MDP. 2006. Levels of nitrate in Swedish lettuce and spinach over the past 10 years. Food Addit Contam. 23:1283–1289.

Muramoto J. 1999. Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. Berkeley (CA): Centre for Agroecology and Sustainable Food Systems, University of California.

Available from: http://www.agroecology.org/people/joji/research/leafnitrate.pdf/
Pegg RB, Shahidi F. 2000. Nitrite curing of meat. The nitrosamine problem and nitrite alternatives. Trumbull (CT): Food & Nutrition Press. p. 269.
Pennington JAT. 1998. Dietary exposure models for nitrates and nitrites. Food Contr. 9(6):385–395.
Sanchez-Echaniz J, Benito-Fernández J. 2001. Methemoglobinemia and consumption of vegetables in infants. Pediatrics. 107:1024–1028.
Schuddeboom LJ. 1993. Nitrates and nitrites in foodstuffs. Strasbourg (France): Council of Europe Press. p. 5–14.
Susin J, Kmecl V, Gregorcic A. 2006. A survey of nitrate and nitrite content of fruit and vegetables grown in Slovenia during 1996–2002. 23:385–390.
Swann PF. 1975. The toxicology of nitrate, nitrite, and N-nitroso compounds. J Sci Food Agricult. 26: 1760–1770.
Tamme T, Reinik M, Roasto M, Juhkam K, Tenno T, Kiis A. 2006. Nitrates and nitrites in vegetables and vegetable-based products and their intakes by Estonian population. Food Addit Contam. 23:355–361.
Tannenbaum SR, Correa P. 1985. Nitrate and gastric cancer risks. Nature. 317:675–676.
Thomson B. 2004. Nitrates and nitrites dietary exposure and risk assessment. Available from: http://www.nzfsa.govt.nz/consumers/food-safety-topics/chemicals-in-food/residues-in-food/consumer-research/nitrite-nitrate-report.pdf/
Van Vliet JJH, Vaessen HAMG, van den Burg G, Schothort RC. 1997. Twenty-four-hour duplicate study 1994; nitrate and nitrite: method development and intake per person per day. Cancer Lett. 114:305–307.
World Health Organization (WHO). 2003. Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consultation. Geneva (Switzerland): WHO. Technical Report Series 916. Available from: http://www.who.int/hpr/NPH/docs/who_fao_expert_report_pdf/
Wolff IA, Wasserman AE. 1972. Nitrates, nitrites and nitrosamines. Science. 177:15–18.
Ximenes MIN, Rath S, Reyes FGR. 2000. Polarographic determination of nitrate in vegetables. Talanta. 51:49–56.
Zhong WK, Hu CM, Wang MJ. 2002. Nitrate and nitrite in vegetables from north China: Content and intake. Food Addit Contam. 19:1125–1129.