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Abstract

The content of nitrates were determined in 1349 samples of vegetables and ready-made food in 2003 - 2004 as a part of the Estonian food safety monitoring programme and the Estonian Science Foundation grant research activities. The results of manufacturers' analyses carried out for internal monitoring were included in the study. The highest mean values of nitrates were detected in dill, spinach, lettuce and beetroot. The mean concentrations were 2936, 2508, 2167 and 1446 mg kg^{-1}, respectively. The content of nitrites in samples was lower than 5 mg kg^{-1}. In total, the mean intake of nitrates by the Estonian population was 58 mg day^{-1}. The mean content of nitrates in vegetable-based infant foods of Estonian origin was 88 mg kg^{-1}. The average daily intake of nitrates by children in the age group of 4 to 6 years was 30 mg. The infants' average daily intake of nitrates from consumption of vegetable-based foods was 7.8 mg.

Keywords: nitrate, nitrite, vegetables, intake

Introduction

Nitrate and nitrite can be found in food as naturally occurring compounds, drinking water and vegetables being substantial sources of nitrate intake. Interest in the dietary intakes of nitrates and nitrites has arisen from the concern about their possible adverse effect on health (Knekt et al.1999; Pegg and Shahidi 2000). An acceptable daily intake

(ADI) of 0 to 3.7 mg kg^{-1} body weight for nitrate and of 0 to 0.06 mg kg^{-1} body weight for nitrite has been established by EU Scientific Committee for Food ((EU Scientific Committee 1995). It has been estimated that vegetables constitute a major source of human exposure to nitrates contributing approximately 80 to 92% of the average daily intake ((Dich et al. 1996).

Levels of nitrates in vegetables differ to a large extent. According to nitrates content the vegetables can be divided into 3 groups:

1. Plants with high nitrate content (> 1000 mg kg^{-1}) – beetroot, lettuce, spinach, other green leaf vegetables and herbs .;
2. Plants with average nitrate content (50 to 1000 mg kg^{-1}) – potatoes and other vegetables;
3. Plants with low nitrate content (0.5 to 50 mg kg^{-1}) – berries, fruits, cereals, pod vegetables.

Nitrate concentration in vegetables 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 (Muramoto 1999; Fytianos and Zagoriannis 1999). Even among different samples of the same vegetable varieties, the range of concentrations can be manifold. The limit concentrations for nitrates have been established in EU for spinach, lettuce and baby foods (European Commission 2002; European Commission 2004).

The nitrate ion has a low level of acute toxicity, but if transformed into nitrite, it may constitute a health problem. Reduction to nitrite may take place in the presence of bacteria or enzyme nitrate reductase, and in contact with metals. Nitrite is unstable at acidic pH values at which it can disproportionate to yield nitrate and nitrogen oxide and/or react with food components including amines, phenols and thiols (Hill 1996). It has been estimated that 5 to 8% of the nitrate from the diet may be reduced to nitrite by the microflora in the oral cavity (Mensinga et al. 2003). Although nitrates and nitrites have been used for centuries, it has only recently been discovered that nitrate is manufactured in mammals by the oxidation of nitric oxide and that the nitrate formed has the potential for disinfecting the food we eat (Benjamin 2000; Archer 2002).

Nitrite in food is primarily considered to be a health problem because its presence both in food and body may lead to the formation of carcinogenic nitrosoamines (JECFA 1996; Vermeer et al. 1998) and the clinical symptom of methemoglobinemia (WHO 1995). 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). It is possible that other factors such as intake of vegetables, fruit and nitrosation inhibitors, or some component of cured meat and salted fish other than N-nitroso compounds could partly be responsible for the observed associations (Eichholzer and Gutzwiller 2003). 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 methemoglobinemia (Sanchez-Echaniz and Benito-Fernández 2001). Processed infant foods may also contain increased levels of nitrites (Hill 1996).

Estonian food safety monitoring programme was initiated in 1998 (Reinik et al. 2001). Concentrations of food additives and contaminants in main food groups are determined annually within the frames of the programme. Obtained data were used for assessment of dietary risks connected with consumption of food additives and contaminants.

Materials and methods

Samples

Altogether, 1349 samples of vegetables and ready-made food were analyzed in 2003 - 2004. Samples were collected mainly at the retail level by food inspection authorities within the frames of state official surveillance and monitoring programme, by researchers within activities of the Estonian Science Foundation grant no. 5416, or were brought to the laboratory by the farmers with the aim of self-control. Approximately 80% of the analyzed vegetables had been grown in Estonia. The minimal amount of vegetable sample was 1 kg. Sample collection covered all seasons. In addition to raw vegetables, vegetable-based baby foods and ready-made soups were analyzed.

Methods of analysis

Nitrate concentrations in vegetables were determined by potentiometric method based on Russian standard GOST 4228-86 (Gosudarstvennõi agropromõšlennõi komitet 1986). Prior to analysis non-edible parts of the sample were removed. In case of small vegetables the whole sample was chopped. Large sample units (e.g. head cabbage) were cut vertically into four pieces, one quarter from each unit was taken for the analysis.

Vegetable juice was prepared using a juice press. Leafy and low juice-containing vegetables were finely chopped. Nitrates were extracted by $KAl(SO_4)_2$ solution and determined potentiometrically by ion-selective electrode. The method has been accredited by Estonian Accreditation Body. Limit of determination was 30 mg kg^{-1} and measurement uncertainty up to 15 % (k=2, norm.) depending on the sample matrix.

The concentration of nitrates and nitrites in infant purees and ready-made food were determined by an HPLC method, based on NMKL (Nordic Committee on Food Analysis) method no. 165, 2000. Nitrates and nitrites were extracted from the samples by hot water. Acetonitrile was added for removal of interfering substances. The filtered solution was injected into a Shimadzu LC10 chromatograph, nitrate and nitrite were separated by Alltech C_{18} column and detected by UV-detector at the wavelength of 205 nm. The limit of quantification for both ions was 5 mg kg^{-1}, measurement uncertainty (U) at the concentration of 100 mg kg^{-1} was 12 mg kg^{-1} (k=2, norm.). The method has been accredited by Estonian Accreditation Body. The Tartu Laboratory of Estonian Health Protection Inspectorate has successfully participated in collaborative study of the method (Merino et al. 2000) and in intercomparison tests.

Method of intake calculation

Different methods can be used for the estimation of additive and contaminant intake from food: food supply data, published tables of the mean consumption of dietary items, duplicate portion analysis, dietary survey among the individuals, probabilistic and worse case screening models. Depending on the method used, intake estimates can differ to a large extent (Kroes et al. 2002).

In this work food consumption data from the Board of Statistics were used in the calculation of average intake for the whole population. Intake of nitrates by children was estimated using the consumption data from kindergarten menus and nutrition recommendations for infants. Consumed amounts of vegetables were reduced, taking into account the effect of peeling, cleaning and removing of non-edible parts. Nitrate concentrations of the commodities were corrected for cooking loss.

Results and discussion

Concentration of nitrates in vegetables

Limit values for nitrate concentration in several vegetables including potato, cabbage, carrot, beetroot, onion, cucumber etc. had been valid in Estonia before joining the EU in 2004. Due to the existing legal limits, a lot of samples were analyzed annually within the framework of official control. Maximum permitted concentrations were exceeded in 17% of the samples, most frequently for cucumber in winter period, spring onion, cabbage, turnip and beetroot. Exceedance of the EU permitted concentrations were not detected, although the nitrate concentration was close to the MRL in one spinach sample.

The results for nitrate analyses in all vegetables are shown in Table I. As seen from the table, the highest mean values of nitrates were detected in dill, spinach, lettuce and beetroot. The mean concentrations were 2936, 2508, 2167 and 1446 mg kg^{-1}, respectively. The lowest mean values of nitrates were detected in tomato, onion and potato, the contents being 41, 55 and 94 mg kg^{-1}, respectively. In vegetables of Estonian

origin, the content of nitrites was lower than 5 mg kg^{-1}- the detection limit of the method. As in Estonia potatoes are consumed in large amounts, the number of the studied samples is the largest. Statistical difference between nitrate content of early, late and stored potatoes was not observed. Average nitrate concentration in imported potatoes was 200 mg kg^{-1}, significantly higher than that of domestic tubers. Similar nitrate concentrations have also been detected in other countries (Table II), with the exception of nitrates content in spinach and potato, according to the data of Chung et al. (2003). The contents of nitrate in spinach grown in Korea and Japan were 1.5 to 2.0 fold higher. The nitrate content in potatoes of Estonian and Finnish origin was 94 and 82 mg kg^{-1}, in those of Korean and Japanese origin it was 452 mg kg^{-1} and 713 mg kg^{-1}, respectively (Penttilä 1995; Chung et al. 2003).

[Insert Table I and II about here]

Leafy vegetables accumulate high amounts of nitrates, concentrations reaching up to 6000 mg kg^{-1}. Nitrate concentration in some salad crops of different varieties during summer and winter seasons were screened by Escobar-Gutierrez (2002): nitrate concentration showed great variability between cultivars and also varieties within one cultivar, exceeding of limit concentrations was more frequent in summer season than in winter. In the present study the nitrate content of lettuce was lower in summer (average 1952 mg kg^{-1}) than in winter (average 3024 mg kg^{-1}), exceeding of limit concentrations was not detected.

Significantly higher nitrate content was found in Italian organically grown green salad and rocket than in those conventionally produced (De Martin and Restani 2003). On the contrary, US investigation (Worthington 2001) states that organic crops contained significantly less nitrates than conventional crops. The review of literature conducted by Heaton (2001) found 14 studies showing lower nitrate content (50%) in organically grown crops and two studies showing insignificant differences.

Concentration of nitrates and nitrites in vegetable-based products for infants

Thirty one samples of canned infant food and 10 carrot juice samples were analyzed for obtaining data for intake calculations. Nitrate contents close to EU limit concentration of 200 mg kg^{-1} were detected in two samples. Nitrate concentration was the highest in the purees containing carrot and pumpkin: 62 to 148 mg kg^{-1} and 124 to 162 mg kg^{-1}, respectively. The highest nitrate content, 251 mg kg^{-1}, was detected in fresh carrot juice, which is frequently fed to babies (Table III). Similar results have been reported in a Spanish study (Hardisson et al. 1996), where nitrate concentrations exceeding the level of 250 mg kg^{-1} were found in baby foods in which the main ingredient was carrot (Table III). The mean contents of nitrates in vegetable-based infant foods of Estonian and Spanish origin were 88 and 92 mg kg^{-1}, respectively. Nitrites were not detected in samples of infant and ready-made food of Estonian origin.

[Insert Table III about here]

Intake calculation

Intake of nitrates has been evaluated, using the data from three different consumption surveys in Estonia (see Tables IV, V and VI). Data were collected from the household

consumption survey made by the National Board of Statistics in Estonia. Data include the number of population and monthly average amounts of consumed vegetables by Estonian population in 2000-2002. Calculations of the mean daily intake of vegetables were made according to the data of the whole population.

In Table IV the amounts of vegetables consumed daily are presented. Consumed amounts of vegetables were reduced, taking into account the effect of peeling, cleaning and removing of non-edible parts. Nitrate concentrations of the commodities were corrected for cooking loss.

The consumption data in the tables were obtained in 2005 from the menu of two kindergartens. During the year, the consumption data according to the menu and amount of supplied vegetables were taken into account. The mean consumed amounts of vegetable-based foods by children in the age group of 1 to 6 years were calculated taking into consideration the average number of children (n=335).

The data for consumption levels in Table V were corrected for cooking and peeling loss for mean nitrate content in mg kg^{-1}. The consumption data in Table VI were calculated on the basis of the infant menu recommended by Estonian paediatricians. The recommended infant menu of the age group of 6 to 12 months should contain 50% of vegetable based foods and 50% of fruit based foods in addition to breast-feeding.

[Insert Tables IV-VI about here]

The average content of nitrates in each vegetable commodity or vegetable based food has been used in all calculations of the intake. The average body weight used for comparison of the results with ADI (Acceptable Daily Intake) was 60 kg. Estonian and foreign products were not differentiated in the survey from the National Board of Statistics of Estonia, both white and red cabbages were included in the consumed amounts of cabbage (Table IV).

The highest intakes of nitrates by Estonian population were obtained from consuming of cabbage and potato: 14 and 12 mg day^{-1}, respectively (Table IV). The following highest intakes were obtained by consuming of cucumber and beetroot: 8.2 and 7.3 mg day^{-1}, respectively. The mean total intake of nitrate per person in Europe ranges between 50 and 140 mg per day and in the USA about 40 to 100 mg per day (Mensinga et al. 2003; Ysart et al. 1999). Mean daily intake of nitrates by Estonian 1- to 6-year-old children was calculated on the basis of the consumption survey from the menus and amounts of supplied vegetables of two kindergartens. Table V indicates the daily intakes of nitrates and the intake percentage from ADI value by children in the age group of 4 to 6 years, consuming vegetable-based foods. The intake of nitrates by 4- to 6- years-old children was 30 mg (40% of ADI), the average body weight of 20 kg was used in the calculations. The obtained results indicated that although the mean nitrate intakes did not exceed ADI, the maximum intakes were several times higher than mean values. The menu of the age group of 1- to 3-years-old was similar to 4- to 6-years-old children group with the difference of 15% less consumed food amount. According to the calculations, the intake of nitrates by this age group was 26 mg day^{-1} (52% of ADI). For calculation of intakes, the average body weight of 15 kg was used. The daily nitrate

intake by Finnish adolescents and children was 47.9 mg day^{-1} (Penttilä 1995). In a Polish study (Wawrzyniak et al. 2003), nitrates intake for 1- to 6-years-old children exceeded ADI twice and the main food source of nitrates were vegetables (90%).

The mean daily intake of nitrate among Estonian infants aged 6 to 12 months was calculated on the basis of the recommended infant menu. Table VI indicates the recommended daily consumption amounts of vegetable-based purees and juices for infants. The mean contents of nitrates in infant foods are presented in Table III with the average content of 88 mg kg^{-1}. Table VI confirms that older infants get higher amounts of nitrates compared to younger ones. For instance, a 6 months old infant gets 4.4 mg and a 12 months old infant 11 mg nitrates per day. An infant's average daily intake of nitrates from consumption of vegetable-based infant foods was 7.8 mg (22% of ADI).

Conclusions

In the present investigation, the mean values and contents of nitrates and nitrites in vegetables and vegetable products were determined. In Estonia for the first time the daily intake of nitrates, obtained from vegetables by children and the whole population, was estimated. According to the EU legislation on food contaminants, exceedance of the permitted limits for nitrates in vegetables and infant food were not detected. The possible reason for low levels of nitrates in vegetables and infant foods was probably related with relatively low use of fertilizers in Estonia. The results showed that cabbage and potato were the main sources of nitrate intake. The mean intake of nitrates from vegetables by Estonian population was 58 mg per day, calculated on the basis of body weight of 60 kg, which made up 26% of ADI value. Nitrate intake by 1- to 6-years-old

children was found to be 28 mg per day (46 % of ADI). The mean nitrates intake from infant food by children aged 6 to 12 months was 7.8 mg per day, calculated on the basis of body weight of 15 kg (22% of ADI). Considering the toxicity of nitrates and the possibility of their transformation to nitrite and carcinogenic N-nitrosoamines, the importance of information on daily intake by children is obvious.

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Table I Nitrate contents of Estonian vegetables in 2003-2005

Commodity	No. of samples	Minimum nitrate content, mg kg^{-1}	Maximum nitrate content, mg kg^{-1}	Average nitrate content, mg kg^{-1}
Potato	449	<30	360	94
Carrot	202	<30	525	148
Cabbage	168	74	1138	437
Beetroot	130	214	3556	1446
Cucumber	130	<30	1236	160
Turnip	58	64	1062	307
Chinese cabbage	36	232	2236	1243
Tomato	25	<30	100	41
Onion	21	30	92	55
Spring onion	15	160	920	477
Lettuce	14	397	3230	2167
Pumpkin	8	<30	445	174
Radish	6	670	1500	1309
Dill	5	2236	3267	2936
Rhubarb	4	55	376	201
Celery	4	256	830	565
Parsley	4	674	1588	966
Strawberry	3	<30	111	55
Cauliflower	3	104	404	287
Zucchini	2	330	511	421
Watermelon	1	95	95	95
Spinach	1	2508	2508	2508
Carrot juice	10	76	251	136
Infant purees	31	26	208	83
Ready-made salads	14	80	474	259
Ready-made vegetable soups	5	588	740	639

Table II Comparison of mean nitrate contents of vegetables (mg kg^{-1}) in different countries (Penttilä 1995; Dejonckheere et al., 1994; Petersen & Stoltze, 1999; Belitz & Grosch, 1999; Chung et al., 2003).

Vegetable commodity	Finland	Great Britain	Belgium	Denmark	United States	Korea	Japan	Germany
Potato	82	150	154	182		452	713	93
Carrots	264	274	278			316	193	232
Lettuce	1835	2330	2782		1489	2430		1489
Chinese cabbage	1057			1859		1740	1040	
Cabbage	607	712		333	451	725		451
Turnip	908							
Tomato	170		36					27
Sweet pepper	140		93					
Cucumber	240	151	344			212	384	
Onion	140	235	59			23		
Beetroot	1800			1505				1630
Green beans	455		585					
Radish		2600	2136			1878	1060	2030
Spinach		2470	2297	1783	965	4259	3560	965
Leek			841	284		56		
Spring onion						436	145	
Garlic						124	455	
Green pepper						76	99	

Table III Comparison of the concentrations of nitrates in vegetable-based infant food

Description of food	Estonia (this study)	Nitrate, mg kg^{-1} Spain (Hardisson et al.1996)	Germany (Belitz and Grosch 1999)
Vegetable purees	19-208		
Vegetable and meat purees	32-148	20-204	
Vegetable and cereal purees	24-162	382	
Carrots, carrot juice	76-251	104	
average	88 (n = 41)	92	81

Table IV Mean daily nitrate intake in Estonia, calculated on the basis of the consumption survey from the National Board of Statistics

Commodity	Consumption, g per day	Mean nitrate content, mg kg^{-1} (cooking and peeling losses taken into account)	Mean nitrate intake, mg per day	% of ADI
Potato	206	57	12	5.3
Cabbage	36	382	14	6.2
Carrot	24	129	3.1	1.4
Beetroot	6.7	1085	7.3	3.3
Turnip	4	269	1.1	0.5
Tomato	21	41	0.9	0.4
Cucumber	51	160	8.2	3.7
Onion	14	55	0.8	0.4
Lettuce	2.8	2167	6.1	2.7
Pumpkin and Zucchini	2.6	260	0.7	0.3
Radish	0.4	1300	0.5	0.2
Ready-made salads	2.9	259	0.8	0.3
Ready-made soups	1.8	639	1.2	0.5
Frozen vegetables	1.6	148	0.2	0.1
Herbs (celery, parsley, dill)	0.9	1489	1.3	0.6
Total	376		58	26

ADI – Acceptable Daily Intake

Table V Mean daily nitrate intake of Estonian children, calculated on the basis of the consumption survey from the menu of kindergartens

Commodity	Consumption, g per day	Mean nitrate content, mg kg^{-1} (cooking and peeling losses taken into account)	Mean nitrate intake, mg per day	% of ADI
Potato	150	57	8.6	11
Cabbage	17	382	6.5	8.6
Carrot	21	129	2.7	3.6
Beetroot	7	1085	7.6	10
Turnip	5.7	269	1.5	2.0
Tomato	4.3	41	0.2	0.2
Cucumber	8.5	160	1.4	1.8
Onion	10	55	0.6	0.7
Spring onion	0.5	477	0.2	0.3
Frozen vegetables	1.6	148	0.2	0.3
Herbs	0.7	1489	1.0	1.4
Total, 4- to 6-years-old children	226		30	40
Total, 1- to 3-years-old children	198		26	52

ADI – Acceptable Daily Intake

Table VI Mean daily nitrate intake of infants, calculated on the basis of the recommended infant menu

Age	Body weight, kg	Recommended consumption of vegetable purees, g	Mean nitrate intake, mg per day	% of ADI
6 months	7.8	50	4.4	15
7 months	8.5	70	6.2	20
8 months	9	80	7.0	21
9 months	9.4	90	7.9	23
10 months	9.8	100	8.8	24
11 months	10.3	110	9.7	25
12 months	10.6	120	11	27
Average	9.3	89	7.8	22

ADI – Acceptable Daily Intake