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Review

Nitrate in vegetables: toxicity, content, intake and EC regulation

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Abstract: Nitrate content is an important quality characteristic of vegetables. Vegetable nitrate content is of interest to governments and regulators owing to the possible implications for health and to check that controls on the content are effective. Nitrate itself is relatively non-toxic but its metabolites may produce a number of health effects. Until recently nitrate was perceived as a purely harmful dietary component which causes infantile methaemoglobinaemia, carcinogenesis and possibly even teratogenesis. Recent research studies suggest that nitrate is actually a key part of our bodies' defences against gastroenteritis. In this review are reported: (1) vegetable classification as a function of nitrate accumulation; (2) vegetable contribution to the total dietary exposure of nitrate; (3) European Commission Regulation No. 563/2002 which sets limits for nitrate in lettuce and spinach; (4) the maximum levels set in some countries for beetroot, cabbage, carrot, celery, endive, Lamb's lettuce, potato, radish and rocket; (5) the results of surveys on the nitrate content of vegetables in Italy and other European countries.

Keywords: nitrate content; nitrate toxicity; vegetable quality; limits for nitrate

INTRODUCTION

Nitrate is a naturally occurring form of nitrogen and is an integral part of the nitrogen cycle in the environment. Nitrate is formed from fertilizers, decaying plants, manure and other organic residues. It is found in the air, soil, water and food (particularly in vegetables) and is produced naturally within the human body.[1–4] It is also used as a food additive, mainly as a preservative and antimicrobial agent.[3,4] It is used in foods such as cheese and cheese products, raw and processed meats, edible casings, processed fish, fish products, spirits and liqueurs.

Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture, vegetables and drinking water may contain higher concentrations of nitrate than in the past.

NITRATE TOXICITY

The presence of nitrate in vegetables, as in water and generally in other foods, is a serious threat to man's health. Nitrate per se is relatively non-toxic,[3,5] but approximately 5% of all ingested nitrate is converted in saliva and the gastrointestinal tract to the more toxic nitrite.[6,7] The only chronic toxic effects of nitrate are those resulting from the nitrite formed by its reduction by bacterial enzymes.[5] Nitrite and *N*-nitroso compounds, which form when nitrite binds to other substances before or after ingestion (for example, the amines derived from proteins), are toxic and can lead to severe pathologies in humans.[8,9] Thus, the assessment of the health risk of nitrate to humans should encompass the toxicity of both nitrite and *N*-nitroso compounds.[8]

Sources of nitrate, nitrite and *N*-nitroso compounds are normally exogenous,[10] but endogenous formation of these compounds has also been demonstrated in both laboratory animals and humans.[2]

The best-known effect of nitrite is its ability to react with haemoglobin (oxyHb) to form methaemoglobin (metHb) and nitrate:

$$NO_2^- + \text{oxyHb}(Fe^{2+}) \longrightarrow \text{metHb}(Fe^{3+}) + NO_3^-$$

As a consequence of the formation of metHb the oxygen delivery to tissue is impaired.[11,5]

Once the proportion of metHb reaches 10% of normal Hb levels, clinical symptoms (from cyanosis—the blue discoloration of the skin due to the presence of deoxygenated blood—through to asphyxia—suffocation) occur. This potentially fatal condition is known as methaemoglobinaemia, or blue baby syndrome.[1,11] Children and adults are far less susceptible to methaemoglobinaemia

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than young infants, because of the induction of methaemoglobin reductase during the physiological post-weaning period. Babies less than three months old are particularly susceptible to methaemoglobinaemia. This is believed to occur because of infants' higher levels of fetal oxyHb still present in the blood, which oxidizes to metHb more readily than non-fetal oxyHb. In addition, infants have less of the reductase needed to reconvert the metHb back to oxyHb, have a proportionately higher intake of nitrate through drinking water by body weight, and have a higher reduction of nitrate to nitrite due to low gastric acidity.[5,12]

Two cases of blue baby syndrome were recently investigated in Wisconsin (USA). Both cases involved infants who became ill after being fed formula that was reconstituted with water from private wells. Water samples collected from these wells during the infants' illnesses contained nitrate N concentrations of 22.9 and 27.4 $mg\,L^{-1}$.[11]

Nitrite as such, and nitrate when reduced to nitrite, may react with amines or amides to form carcinogenic *N*-nitroso compounds.[5] Nitrosation can occur mainly in two situations: (1) during storage and ripening of the food product[1] and (2) in the stomach from the action of salivary nitrite produced through enzymatic reduction of endogenous or exogenous nitrate.[13] Since the discovery of the carcinogenicity of *N*-nitrosodimethylamine in rats by Magee and Barnes,[14] *N*-nitroso compounds have been shown to be carcinogenic in more than 40 animal species.[2] These include mammals, birds, reptiles and fish, and there is no reason to suspect that human beings are uniquely resistant.[15]

Several authors have suggested that the risk for the development of stomach cancer is positively correlated with three factors: (1) the nitrate level of drinking water, (2) the urinary excretion of nitrate and (3) the occurrence of atrophic gastritis.[8] Epidemiological studies have not provided any evidence that there is an increased risk of cancer related to high nitrate intake from sources other than vegetables.[2] In some cases studies revealed a negative correlation between nitrate intake and gastric cancer,[2,16,17] because vegetables are an excellent source of vitamins, minerals and biologically active compounds.[18,19]

Some years ago, Vermeer *et al.*[20] showed the endogenous formation of carcinogenic *N*-nitroso compounds (*N*-nitrosodimethylamine and *N*-nitrosopiperidine) after intake of nitrate at the acceptable daily intake (ADI, see below) level in combination with a fish meal rich in amines as nitrosatable precursors. The vegetables used in the research (cauliflower, peas, carrots and green beans) were low in nitrate, and their mean vitamin C content (an anti-carcinogenic agent) was approximately 170 $mg\,kg^{-1}$ vegetables. Thus, the amount of vitamin C (and other antioxidants) in these vegetables appeared insufficient to prevent nitrosamine formation.[20] The same Danish research group has shown that nitrate can interfere with iodine uptake by the thyroid, resulting in hypertrophy of the thyroid, the gland responsible for many of the body's endocrine and hormonal functions.[21]

More recently, another research group has shown a positive relationship between the incidence of childhood-onset insulin-dependent diabetes mellitus and levels of nitrate in drinking water.[22] Their findings suggest that the threshold for the effect is 15 $mg\,L^{-1}$ of NO_3 (less than one-third of the EC limit for nitrate in drinking water), which is considered both worrying[23] and puzzling.[24]

Recent research suggests that dietary nitrate may have beneficial effects, based on the hypothesis that nitric oxide (NO) formed in the stomach from dietary nitrate has antimicrobial effects on gut pathogens and a role in host defence.[12,25,26]

The potential beneficial effects of nitrate have been the subject of limited research; however, there was enough evidence in several areas including prevention of microbial infections, reduction of hypertension and cardiovascular diseases, and reduction in the risk of gastric cancer, to lead two researchers to publish a paper entitled 'Are you taking your nitrate?'[24]

Finally, it is important to cite the text 'Nitrate and man: toxic, harmless or beneficial?' which, as suggested by the title, is a broad-ranging review of the role of nitrate in human health.[27] The authors critically review the evidence relating to the reported adverse effects of nitrate and note that a plausible hypothesis (the toxicity of nitrate) has been transformed into a practically sacrosanct dogma, in spite of the lack of proof.[27]

However, an intake of vegetables and consumption of drinking water with such a high nitrate content that the ADI is exceeded for a prolonged period should be avoided.[28] Thus, in order to gain as much as possible from the indisputable benefits of vegetables, a reduction in nitrate levels is highly desirable for consumers and probably profitable for farmers.[29]

ACCEPTABLE DAILY INTAKE

The concept of ADI is defined by the Joint Expert Committee of the Food and Agriculture (JECFA) Organization of the United Nations/World Health Organization (WHO) for substances intentionally added to food or for contaminants (pesticides, herbicides and fertilizers).[3,4] In view of the well-known benefits of vegetables and the lack of data on the possible effects of vegetable matrices on the bioavailability of nitrate, JECFA considered it to be inappropriate to compare exposure to nitrate from vegetables with ADI or to derive limits for nitrate in vegetables directly from it.[3] In the absence of an appropriate alternative approach in the literature the consequences of nitrate intake exceeding the ADI are discussed.

The JECFA and the European Commission's Scientific Committee on Food (SCF) have set an ADI for NO_3 of 0–3.7 $mg\,kg^{-1}$ bodyweight.[3,4,30–32]

Table 1. Estimated intakes of NO_3 from sources other than food additives at the global level (after Hambridge[34])

Regional diet	Intake ($mg\ day^{-1}$)	ADI[a] ($\mu g\ mg^{-1}$)	Major contributors to total intake ($\mu g\ mg^{-1}$)			
			Vegetables	Water	Cereals	Fruit
Middle Eastern	40	200	650	200	100	50
Far Eastern	28	100	450	300	150	100
African	20	100	300	400	150	100
Latin American	55	250	650	150	50	100
European	155	700	900	50	<50	50

[a] Based on 60 kg body weight.

The USA Environmental Protection Agency (EPA) Reference Dose (RfD) for nitrate is 1.6 mg nitrate nitrogen kg^{-1} bodyweight (bw) per day (equivalent to about 7.0 mg $NO_3\ kg^{-1}$ bw per day).[5]

The JECFA and SCF have proposed an ADI for NO_2 of 0–0.07[8,9] and 0–0.06 mg $NO_2\ kg^{-1}$ bw[32], respectively, while the EPA has set an RfD of 0.1 mg nitrite nitrogen kg^{-1} bw per day (equivalent to 0.33 mg $NO_2\ kg^{-1}$ bw per day).[5]

The SCF retains that the ADIs are applicable to all sources of dietary exposure.[30,31]

NITRATE INTAKE

The three main sources of nitrate intakes are vegetables, water and cured meat.[1,33]

Vegetables constitute the major dietary source of nitrate, generally providing from 300 to 940 mg g^{-1} of the daily dietary intake (Tables 1 and 2). Their contribution to nitrite intake is low, and in fact lower than that from cured meat products.[1,10,33] Nitrite is found in plant foodstuffs, typically 1–2 mg kg^{-1} of fresh vegetable weight.[46] Potatoes, however, can contain up to 60 mg $NO_2\ kg^{-1}$.[46] Higher amounts of nitrite are found in contaminated food or in broken vegetable tissues in food stored for several days at room temperature.[33]

Table 2. Estimate of nitrate daily intake (drinking water not included) and contribution of vegetables in various countries

Country	NO_3 (mg $person^{-1}$)	Vegetable contribution[c] ($\mu g\ mg^{-1}$)	Reference
Belgium	148[a]	93	Dejonckheere *et al*.[35]
European Union	18–131[b]	100	SCF[32]
Finland	77	92	Dich *et al*.[36]
France	121	85	Cornée *et al*.[37]
Germany	68	72	Selenka and Brand-Grimm[38]
Italy	149	90	CSS[39]
Poland	65–85	ND	Borawska *et al*.[40]
Spain	60	ND	ACAPV[41]
Sweden	50	ND	Jagerstad and Nilsson[42]
The Netherlands	52	ND	Ellen *et al*.[43]
UK	95	94	Knight *et al*.[44]
USA	73	90	NRC[45]

[a] Included only vegetables and fruit.
[b] Included only vegetables.
[c] Not detected.

Compared with the current ADIs, the ingestion of only 100 g of raw vegetables with a nitrate concentration of 2500 mg kg^{-1} will already lead to an intake of 250 mg NO_3. Consuming this item alone, for a person of 60 kg, would exceed the ADI for nitrate by 13%. Calculating in the partial conversion of nitrate to nitrite (5%) after such consumption, the current SCF ADI for nitrite (0.06 mg kg^{-1} bw) would be exceeded by 247%.

A statistical exposure model has shown that in the adult population in the Netherlands 15% had daily intakes regularly exceeding the ADI;[47] in young children this may rise to 45%.[47]

In its last report, the SCF[32] reviewed from a public health standpoint the presence of nitrate in foodstuffs in general, and vegetables in particular, and stated that the total intake of nitrate is normally well below the acceptable daily intake (Table 2). The major sources are potatoes and lettuce, the first because they are vegetables consumed in the largest quantity mainly (Table 3), the latter due to its high nitrate content.[32] The SCF[31,32] recommended continuation of efforts to reduce exposure to nitrate via food and water since nitrate can be converted into nitrite and nitrosamines, and urged that good agricultural practices are adopted to ensure nitrate levels are as low as reasonably achievable.

In previous research, nitrate intake from vegetables was estimated using the data on average daily per capita consumption of vegetables provided by the Italian National Institute of Nutrition[49] and on average nitrate content from the research work: nitrate daily intake from vegetables was 71 mg d^{-1}; over 300 mg g^{-1} of nitrate intake was derived from the consumption of lettuce and Swiss chard.[50]

Dietary exposures to nitrate for vegetarians are very similar to those of other consumers and are below the ADI. The average dietary exposure of the vegetarians in a UK study was 83 mg d^{-1} and the highest nitrate exposure was 209 mg d^{-1}.[51,52]

NITRATE CONTENT IN VEGETABLES

There are several factors affecting NO_3 uptake and accumulation in vegetable tissues, e.g. genetic

Table 3. Food consumption and nitrate daily intake in some countries of European Union (after Schuddeboom[48])

Food group	Country	Food consumption ($g\ person^{-1}$)	Average concentration ($mg\ kg^{-1}$ fm)	NO_3 intake ($mg\ person^{-1}$)	Contribution to nitrate intake ($\mu g\ mg^{-1}$)
Potato	Germany	112	93	10.5	113
	Denmark	166	80	13.3	185
	The Netherlands	131	60	7.9	66
	UK	160	120	19.0	339
Other vegetables	Germany	73	721	52.6	564
	Denmark	114	440	50.2	697
	The Netherlands	150	800	120.0	857
	UK	162	136	22.0	393
Fruit	Germany	101	70	7.1	76
	Denmark	120	30	3.6	50
	The Netherlands	125	20	2.5	18
	UK	91	25	2.3	41

Table 4. Classification of vegetables according to NO_3 content ($mg\ kg^{-1}$ fm)

Very low (<200)	Low (200–500)	Middle (500–1000)	High (1000–2500)	Very high (>2500)
Artichoke	Broccoli	Cabbage	Celeriac	Celery
Asparagus	Carrot	'Cima di rapa' (broccoli rab)	Chinese cabbage	Chervil
Broad bean	Cauliflower	Dill	Endive	Cress
Brussels sprouts Eggplant	Cucumber	'Radicchio'	Escarola	Lamb's lettuce
Garlic	Pumpkin	Savoy cabbage	Fennel	Lettuce
Onion	'Puntarelle' chicory	Turnip	Kohlrabi	Radish
Green bean			Leaf chicory	Red beetroot
Melon			Leek	Rocket
Mushroom			Parsley	Spinach
Pea				Swiss chard
Pepper				
Potato				
Summer squash				
Sweet potato				
Tomato				
Watermelon				

factors, environmental factors (atmospheric humidity, substrate water content, temperature, irradiance, photoperiod) and agricultural factors (nitrogen doses and chemical forms, availability of other nutrients, use of herbicides, etc.).[53–55]

Of the factors studied, nitrogen fertilization and light intensity have been identified as the major factors that influence nitrate content in vegetables.[56] In particular, light intensity and nitrate content in soil before or at harvest are known to be critical factors in determining nitrate levels in spinach[53] or other leafy vegetables.[55,57]

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 (Table 4).

Nitrate content can vary also within species, cultivars and even genotypes with different ploidy.[58]

The differing capacities to accumulate nitrate can be correlated with differing location of the nitrate reductase activity,[59–61] as well as to differing degree of nitrate absorption and transfer in the plant.[53,62]

Nitrate content differs in the various parts of a plant.[50] Indeed, the vegetable organs can be listed by decreasing nitrate content as follows: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed.[50,63]

The highest nitrate-accumulating vegetable is rocket,[50] a leafy vegetable popular in the Mediterranean region.[55,64,65] A number of species of the Brassicaceae family are grouped under the name of rocket belonging to the *Eruca* Miller and *Diplotaxis* DC. genera. *Eruca* is present in both wild and cultivated forms; *Diplotaxis* is known as a wild type. In *Diplotaxis*, two surveys carried out in Italy show NO_3 content reaches up to 9300 $mg\ kg^{-1}$.[50,66]

Rocket absorbs NO_3 very quickly[64,65] and NO_3 concentration in leaves can be much higher than in the growth medium. With 1 $mmol\ L^{-1}$ NO_3 nitrogen in a hydroponic nutrient solution, NO_3 accumulation ratio (expressed as the ratio between the concentration in leaves and in nutrient solution) was, respectively, 55 for *E. vesicaria* and 101 for *D. tenuifolia*.[67]

Soil-less systems offer a clear advantage to traditional ones in the management and control of plant mineral uptake during the various phases of the growing cycle. One advantage of these growing systems is that they can be used to produce vegetables with low nitrate accumulation.[55,65,68]

LIMITS TO MAXIMUM LEVELS OF NITRATE IN VEGETABLES

To protect public health in response to the SCF's considerations of nitrate in food,[30–32] in 1997 the European Member States agreed an EC Regulation setting limits for nitrate in lettuce and spinach (EC Regulation No. 194/97).[69] The main purpose of this EC Regulation was to harmonize limits for nitrate in these vegetables, as the different national limits set by some Member States were causing trade difficulties across the European Union.

On 2 April 2002 the European Commission amended EC Regulation No. 194/97 (already amended with some periphrasis from EC Regulation No. 864/1999[70] and 466/2001[71]) and adopted EC Regulation No. 563/2002.[72] The maximum levels set by this Regulation are summarized in Table 5. The limits vary according to season, with higher nitrate levels permitted in crops grown in winter compared with those grown in the summer. Lower limits are fixed for open-grown lettuce than for lettuce grown under glass, and in order to allow effective control the limits set for open-grown lettuce should apply also to lettuce grown under glass in the absence of precise labelling. Lower limits are fixed for 'iceberg' than other types of lettuce.

The differences between nitrate levels in different varieties have been most extensively studied in relation to lettuce where open leaf varieties generally have higher nitrate concentrations than tight-headed varieties such as iceberg.[32]

EC Regulation No. 563/2002 states that 'in some regions nitrate levels are reported to be frequently higher than those set in the Annex of Regulation (EC) No 466/2001, although the general trend shows that the levels of nitrate in lettuce are decreasing'.[72] The levels of nitrate in spinach show no clear trend for reduction. So, 'some Member States need to maintain the established transitional period to authorise the placing on the home market of lettuce and/or spinach grown and intended for consumption in their territory'.[72] This derogation requires annual monitoring to be carried out to demonstrate that nitrate levels in these crops are acceptable on public health grounds and that growers follow a 'code of good agricultural practice'.[72]

The UK is currently applying this derogation for both lettuce and spinach along with Ireland. A derogation for spinach presently applies in Finland, Denmark and the Netherlands. However, maximum limits do apply to lettuce and spinach imported from Member States and third countries. The derogation for lettuce ended on 1 January 2005. An extension of this derogation is currently under consideration within the EU. The derogation for spinach is currently being reviewed.[72]

Nevertheless, no official method has been published in EU legislation and nitrate levels in vegetables are generally assayed by modifying the protocols used for other foods.

Limits to maximum levels of nitrate for trade in other vegetables are set in some European countries (Table 6). For potato, several countries have put forward the proposal of 'guidelines' for nitrate content (in Germany, for instance, only tubers with less than 200 mg kg^{-1} fresh matter (fm) are accepted), while in Poland there is a maximum limit of 183 mg kg^{-1} fm.[73]

Rocket and other Italian export vegetable (e.g. potato) sales contracts include very strict clauses, for instance with Switzerland and Germany. Namely, nitrate content for rocket is required not to exceed 2.5–4.0 g kg^{-1} fm, which is a very strict threshold that is difficult to respect on account of the high accumulation of nitrate in rocket, even when reduced amounts of nitrate are used in its cultivation.[74]

No nitrate standards for vegetables have been introduced in the USA. In China, a suggested maximum level of nitrate in vegetables of 3100 mg kg^{-1} has been established.[75]

Table 5. Maximum levels (limits) for the nitrate (mg kg^{-1} fm) in lettuce and spinach according to European Commission Regulation (EC) No. 563/2002[72]

Product	Harvest period	NO_3
Fresh spinach[a] (*Spinacia oleracea* L.)	Harvested 1 November to 31 March	3000
	Harvested 1 April to 31 October	2500
Preserved, deep-frozen or frozen spinach		2000
Fresh lettuce (*Lactuca sativa* L.) (protected and open-grown lettuce) excluding 'iceberg' type	Harvested 1 October to 31 March:	
	— lettuce grown under cover	4500
	— grown in the open air	4000
	Harvested 1 April to 30 September:	3500
	— lettuce grown under cover	2500
	— lettuce grown in the open air	
'Iceberg' type lettuces	Lettuce grown under cover	2500
	Lettuce grown in the open air	2000

[a] The maximum levels for fresh spinach do not apply for fresh spinach subject to processing and which is directly transported in bulk from field to processing plant.

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