A survey of nitrate and oxalate content in fresh vegetables

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Abstract: A survey of nitrate (NO_3^-) and oxalate ($(COO^-)_2$) content in fresh vegetables was conducted in Bari (Italy) over 15 months (from March 1994 to May 1995). A total of 327 samples (edible portions and related sub-samples) were taken from 26 different vegetable types on the wholesale fruit and vegetable market. The data revealed that leaf vegetables (namely rocket, celery, parsley and spinach) contained higher levels of nitrate than bulb, root, shoot, inflorescence and tuber vegetables. Higher oxalate levels were found in spinach and Swiss chard. Based on consumption data for the whole population provided by the National Institute of Nutrition, daily nitrate intake from vegetables was calculated to be 71 mg. Over 30% of nitrate intake was derived from the consumption of lettuce and Swiss chard.

Keywords: nitrate content; vegetables; nitrate intake; soluble oxalate

INTRODUCTION

Despite the undoubted contribution of vegetables to human health, due to their low energy value and high content of dietary fibre, vitamins and minerals, consumers are worried about some cultivation techniques which might reduce the quality and safety of productes, eg the presence of pesticide residues[1] or nitrate content.[2]

The presence of nitrate in vegetables, as in water and generally in other food products, is a serious threat to man's health, although recent research has shown that nitrate also has beneficial effects on health related to its role in the body's mechanism against pathogenic micro-organisms.[3] The harmful effects of nitrate are related not so much to its toxicity, which is low, but to the dangerous compounds that are synthesised in the organism. Indeed, the most serious danger comes from nitrite which is produced by nitrate reduction and which can lead to methaemoglobinemia or form nitrosamines and nitrosamides by reacting with amines and amides, whose carcinogenic action is well known.[4,5]

In evaluating nitrate and nitrite, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) set as 0–3.65 mg kg^{-1} body weight the acceptable daily intake (ADI) for (NO_3^-).[6] Subsequently, the European Communities' Scientific Committee for Food (SCF) also set an ADI for (NO_3^-) of 0–3.65 mg kg^{-1} body weight.[7] Compared with this ADI, the ingestion of only 100 g of raw vegetables with a nitrate concentration of 2190 mg kg^{-1} fresh matter (FM) corresponds to the whole nitrate ADI for a person of 60 kg—though it must be observed that some components of vegetables (eg ascorbic acid, phenols, etc) have been reported to inhibit the toxic effects of nitrites.[4,5]

Since 15 February 1997 the concentration of nitrate in spinach must not exceed 3000 and 2500 mg kg^{-1} FM for crops harvested from 1 November to 31 March and from 1 April to 31 October respectively (Commission Regulation (EC) 194/97, published in the Official Journal of the European Community L31/48 of 1 February 1997). For packaged, frozen or deep-frozen spinach the limit is 2000 mg kg^{-1}. For lettuce the following limits are fixed: 4500 and 3500 mg kg^{-1} for crops harvested from 1 October to 31 March and from 1 April to 30 September respectively. For lettuce grown in the open the limit is fixed at 2500 mg kg^{-1} for crops harvested from 1 May to 31 August.

Some cultivated vegetables, namely those belonging to the *Chenopodiaceae* group, accumulate large amounts of oxalate as well as nitrate. Oxalate is a common plant component considered to be an anti-nutrient as well as a toxin;[8] indeed, it can render some mineral nutrients unavailable by binding them to form insoluble salts which are not absorbed by the intestine.[9]

In the present study we report a survey of nitrate and soluble oxalate concentration in retail fresh vegetables based on the consumed edible portion (leaf, stem and laminae, stem, roots, etc) and an assessment of daily nitrate intake from vegetable consumption.

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Table 1. Edible plant portions and related portions considered for nitrate and oxalate content

Vegetable	*Edible portion*	*Sub-sample*	*Sampling date*[a]								
			1	2	3	4	5	6	7	8	9
Beetroot (*Beta vulgaris* L var *vulgaris*)	Root	—	•	•	•	•					
Broccoli (*Brassica oleracea* L var *italica* Plenck)	Inflorescence and uppermost leaves	—				•				•	•
Broccoli raab or rappini (*Brassica rapa* L)	Inflorescence and uppermost leaves	—	•	•	•	•	•	•	•		
Cabbage (*B oleracea* L var *capitata* L)	Leaves	—								•	
Carrot (*Dacus carota* L var *sativus* (Hoffm) Thell)	Root	—		•		•	•	•			
Cauliflower (*B oleracea* L var *botritys* L)	Fleshy inflorescence	—								•	
Celery (*Apium graveolens* L var *dulce* (Mill) Pers)	Leaf stalks (petioles)	Inner and outer leaflets; inner and outer petioles	•	•	•	•	•	•	•		
Chicory (*Cichorium intybus* L)[b]	Leaves and stems (asparagus)	Inner and outer leaves	•	•	•	•	•	•			
Endive (*C endivia* L var *crispum* Hegi)	'Head' (leaves)	—				•				•	•
Fennel (*Foeniculum vulgare* Mill var *azoricum* Mill Thell)	'Bulb'	Inner and outer leaf base	•	•		•	•	•	•		•
Garlic (*A sativum* L)	Bulb	—								•	
Green onion (*Allium cepa* L)	Leaf sheath	—	•			•	•		•		
Kohlrabi (*B oleracea* L var *gongylodes* L)	Shoot	—					•			•	
Lettuce (*Lactuca sativa* L)[c]	'Head' (leaves)	Inner and outer leaves	•	•	•	•	•	•	•		
Onion (*A cepa* L)	Bulb	—	•		•	•	•	•		•	
Parsley (*Petroselinum crispum* (Mill) Nyman ex Aw Hill)	Leaves	Blades and petioles			•	•	•	•	•		•
Potato (*Solanum tuberosum* L)	Tuber	—		•				•			
'Radicchio' (*C intybus* L)	'Head' (leaves)	—								•	•
Radish (*Raphanus sativus* L)	Root and leaves	—	•	•	•	•			•		
Rocket (*Eruca vesicaria* (L) Cav subsp *sativa* (Mill) Thell)	Leaves	—	•	•	•	•		•	•		
Savoy cabbage (*B oleracea* L var *sabauda* L)	Leaves	Inner and outer leaves				•	•	•			•
Spinach (*Spinacia oleracea* L)	Leaves	Blades and petioles	•	•	•	•	•	•	•		
Sweet potato (*Ipomea batatas* (L) Lam)	Root	—				•		•			
Swiss chard (*B vulgaris* L var *cicla* L)	Leaves	Petioles and blades; midrib, petiole and rest of leaf	•	•	•	•	•	•	•		
Tassel hyacinth (*Leopoldia comosa* (L) Parlatore)	Bulb	—	•			•					

[a] 1, 24/03/94; 2, 15/04/94; 3, 10/6/94; 4, 13/10/94; 5, 16/12/94; 6, 28/01/95; 7, 16/02/95; 8, 25/02/95; 9, 12/05/95.
[b] Includes leaf chicory, head chicory and asparagus chicory.
[c] Includes the group *capitata* (butterhead type), *crispa* (crisphead type) and *longifolia* (Romaine type).

MATERIALS AND METHODS

The survey was conducted over 15 months (from March 1994 to May 1995) at the Fruit and Vegetable Wholesale Market of Bari where over 50 000 t year^{-1} of vegetables from Southern Italy have been sold in the last 15 years. The types of vegetables, the relevant edible portions and how they were divided for the analysis, as well as the sampling dates, are reported in Table 1. Fruits were not considered in the survey owing to their recognised low content of nitrate.[10]

A total of 327 samples were analysed, each corresponding to 200–1000 g of each vegetable at periods of their greatest consumption. For each sample only the edible portion, prepared according to common household practice (withdrawal of outermost leaves), was used in tests.

Fresh matter (FM) and dry weight (determined by drying fresh samples in a thermoventilated oven at 65 °C to a constant weight) of the edible portion or of the single portions into which it was divided were determined. Material was then finely ground and used for quantitative chemical analyses of nitrate (NO_3^-) and soluble oxalate ($(COO^-)_2$) using ion chromatography on aqueous extracts of vegetables.[11,12] One analysis was performed for each vegetable part sample. The anions, previously extracted at 25 °C by shaking 0.5 g of dry sample for 30 min in 50 ml of carbonate (1.8 mM) and sodium bicarbonate (1.7 mM) solution, were determined by a Dionex QIC model (Dionex Corp, Sunnyvale, CA) ion chromatograph, using a conductivity detector, guard column IonPac AG4A and analytical column IonPac AS4A.[13,14] The eluent, fluxed at 2 ml min^{-1}, was the same carbonate–sodium carbonate solution used for extraction.

Following our laboratory quality control programme, four spiked analyses were performed for

Table 2. Number of samples, mean content and standard deviation of dry matter and soluble oxalate ($(COO^-)_2$) in vegetable edible portions

	Dry matter			*Oxalate*			
Vegetable	*Total samples*	*Mean ($g\,kg^{-1}$ FM)*	*SD*	*Total samples*	*Mean ($mg\,kg^{-1}$ FM)*	*SD*	*Range ($mg\,kg^{-1}$ FM)*
Asparagus chicory	10	6	8	10	6	4	ND–146
Leaves	8	7	9	8	ND	–	–
Stems	8	6	10	8	116	6	51–217
p-value		NS			NS		
Beetroot	6	9	16	6	749	251	540–1088
Broccoli	4	124	11	4	5	5	ND–110
Broccoli raab	10	101	18	10	1	2	ND–60
Cabbage	4	102	30	3	ND	–	–
Carrot	9	9	24	7	120	5	34–200
Cauliflower	4	9	7	4	ND	–	–
Celery	8	6	13	6	5	7	ND–204
Outer petioles	11	4	7	7	1	2	ND–69
Inner petioles	11	5	14	7	155	238	ND–682
p-value		<0.05			NS		
Petioles	8	5	11	6	7	103	ND–268
Leaftlets	8	104	8	6	ND	–	–
p-value		<0.001			NS		
Endive	5	6	18	5	2	3	ND–76
Fennel	16	6	8	11	124	4	66–199
Garlic	4	322	16	4	ND	–	–
Green onion	3	9	12	3	ND	–	–
Kohlrabi	3	6	12	3	ND	–	–
Leaf chicory	8	7	23	8	ND	–	–
Lettuce				16	ND	–	–
Butterhead type	4	5	8	2	ND	–	–
Crisphead type	9	4	5	6	ND	–	–
Romaine type	3	5	6	1	ND	–	–
p-value		NS					
Onion	6	9	8	6	ND	–	–
Parsley	8	152	18	8	5	8	ND–244
Blades	3	180	12	3	5	8	ND–152
Petioles	3	107	14	3	215	232	ND–482
p-value		<0.01			NS		
Potato	3	157	13	3	4	7	ND–127
'Radicchio'	3	6	5	3	ND	–	–
Radish							
Leaves	6	8	13	6	ND	–	–
Root	6	5	7	6	ND	–	–
p-value		<0.001					
Rocket	10	127	15	10	ND	–	–
Savoy cabbage	2	142	6	2	1	1	ND–21
Spinach	13	9	22	12	5426	2735	2309–10 108
Blades	10	9	12	10	5829	1781	2264–8164
Petioles	10	7	22	9	1589	1115	408–3791
p-value		<0.05			<0.001		
Sweet potato	3	326	6	3	ND	–	–
Swiss chard	16	6	11	12	2077	945	1029–3890
Blades	11	7	10	8	3323	1317	1678–6031
Petioles	11	5	12	8	557	245	233–885
p-value		<0.001			<0.001		
Midribs and petioles	5	4	9	4	722	513	297–1345
Rest of leaves	5	8	14	4	4258	2107	2108–6654
p-value		<0.01			<0.05		
Tassel hyacinth	3	235	17	3	ND	–	–

ND, not detected (the limit of determination for oxalate was $5\,mg\,kg^{-1}$); NS, not significant for $p = 0.05$.

each sampled vegetable. On average, recoveries ranged from a minimum of 96.7% (radish) to a maximum of 103.2% (rocket) for nitrate and from a minimum of 94.6% (beetroot) to a maximum of 105.2% (Savoy cabbage) for oxalate.

Nitrate intake from vegetables was estimated using

Table 3. Number of samples, mean and interval range (min–max) of nitrate (NO_3) content in vegetable edible portions

Edible portion	Vegetable	Samples	Mean[a] (mg kg^{-1} FM)	Range (mg kg^{-1} FM)
Bulb	Garlic	4	34	ND–137
	Onion	4	32	5–115
	Tassel hyacinth	3	53	ND–100
Inflorescence	Broccoli	4	154	ND–440
	Broccoli raab	10	905	321–1705
	Cauliflower	4	202	143–354
Leaf	Cabbage	4	400	8–929
	Celery	6	1678	1009–2163
	Endive	5	224	51–698
	Fennel	11	363	107–769
	Green onion	3	410	69–1046
	Leaf chicory	8	1452	446–2284
	Lettuce			
	Butterhead type	4	1089a	672–1745
	Crisphead type	9	581b	428–810
	Romaine type	3	1241a	684–1766
	Parsley	8	1150	366–1851
	'Radicchio'	3	12	ND–24
	Rocket	10	2597	963–4305
	Savoy cabbage	2	29	26–32
	Spinach	13	1845	547–3350
	Swiss chard	13	2363	1299–4220
Root	Beetroot	6	1727	1023–2414
	Carrot	9	195	28–394
	Radish	6	2067	1117–2993
	Sweet potato	3	54	ND–161
Shoot	Asparagus chicory	10	498	167–889
	Kohlrabi	3	1769	1551–2046
Tuber	Potato	3	81	ND–179

ND, not detected (the limit of determination for nitrate was 5 mg kg^{-1})
[a] Means followed by the same letter are not significantly different ($p < 0.05$).

the data on average daily per capita consumption of vegetables provided by the National Institute of Nutrition[15] and on average nitrate content from the present research work for examined vegetables and from the Italian literature for those not considered in the present study.

The comparison between dry matter, oxalate and nitrate in the different portions into which the edible part was divided was carried out by Student's *t* and variance analysis.

RESULTS

Based on the dry matter content (Table 2), the vegetables considered in this research can be classified into four groups—group I (>200 g kg^{-1} FM): garlic, tassel hyacinth and sweet potato; II (150–200 g kg^{-1} FM): parsley and potato; III (100–150 g kg^{-1} FM): broccoli, broccoli raab, cabbage, rocket and Savoy cabbage; IV (<100 g kg^{-1} FM): green onion, Swiss chard, leaf chicory, endive, fennel, lettuce, 'radicchio', celery and spinach among the leafy vegetables, and beetroot, carrot, cauliflower, asparagus chicory, kohlrabi, onion and radish among the other vegetables.

Nitrate content in spinach was higher in autumn–winter than in spring (2580 compared with 1622 mg kg^{-1} FM). For other vegetable species no significant differences were recorded for nitrate accumulation over the seasons.

Based on the classification of Corré and Breimer,[10] nitrate content of leaf vegetables ranked over 2500 mg kg^{-1} FM in rocket; between 1000 and 2500 mg kg^{-1} FM in celery, chicory, lettuce, leaf beet, parsley and spinach; between 200 and 500 mg kg^{-1} FM in green onion, cabbage, endive and fennel; and below 200 mg kg^{-1} FM in Savoy cabbage and 'radicchio' (Table 3).

Among the lettuce varieties under study the crisphead type accumulated about half the nitrate found in the butterhead and Romaine types (Table 3).

Inner leaves accumulated less nitrate than outer ones in lettuce and 'head chicory'; in parsley and spinach, leaf blade accumulated less nitrate than petiole. In Swiss chard, petiole accumulated more nitrate than blade, which in turn accumulated more than midrib (Fig 1).

Celery inner petioles accumulated less nitrate than outer ones, but the difference was not significant between petioles and leaflets (Fig 1).

Beetroot, kohlrabi and radish accumulated less than 2500 mg kg^{-1} nitrate; broccoli raab between 500 and 1000 mg kg^{-1}; asparagus chicory, broccoli and cauli-

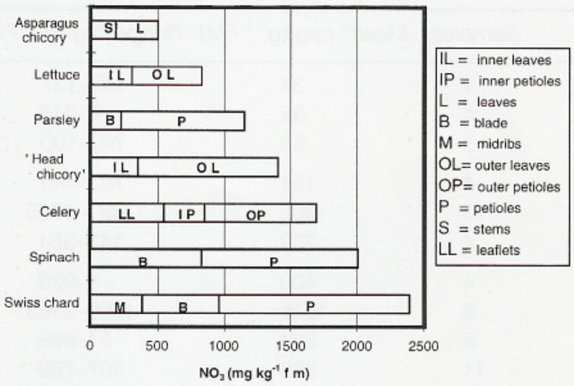

Figure 1. Nitrate content of edible portion sub-samples of several vegetables.

flower between 200 and 500 mg kg^{-1}; and carrot, garlic, tassel hyacinth, onion, sweet potato and potato less than 200 mg kg^{-1} (Table 3).

Asparagus chicory stems accumulated less nitrate than leaves (Fig 1). Radish leaves and root accumulated the same amount of nitrate.

The vegetables considered in the present study can be divided into four groups (Table 2) according to their content of soluble oxalate (mg kg^{-1} FM)—I (>5000): spinach; II (1000–5000): Swiss chard; III (200–1000): beetroot; IV (<200): carrot, broccoli, Savoy cabbage, asparagus chicory, broccoli raab, fennel, endive, potato, parsley and celery. Garlic, cauliflower, cabbage, kohlrabi, leaf chicory, onion, green onion, tassel hyacinth, lettuce, 'radicchio', radish and rocket did not show detectable oxalate.

Spinach and Swiss chard petioles accumulated less oxalate than blades (73 and 83% respectively). In the latter species, midrib and petiole oxalate content was only 17% compared with the other part of leaf (Table 2). The portions into which celery, fennel and parsley were divided did not provide significant differences (Table 2).

Based on the average vegetable consumption in Italy, daily percapita intake of nitrate from vegetables was approximately 71 mg. The most important sources of nitrate were lettuce, Swiss chard and beetroot, which together accounted for 47% of daily intake (Table 4).

DISCUSSION AND CONCLUSIONS

The vegetables accumulating more nitrate belong to the families of *Chenopodiaceae* (beetroot, Swiss chard and spinach), *Brassicaceae* (cabbage, broccoli, broccoli raab, cauliflower, kohlrabi, radish, rocket and Savoy cabbage), *Apiaceae* (carrot, celery, fennel and parsley) and *Asteraceae* (asparagus chicory, endive, lettuce, leaf chicory and 'radicchio'). *Convolvulaceae* (sweet potato), *Solanaceae* (potato) and *Liliaceae* (garlic, onion, green onion and tassel hyacinth) contained the lowest levels of nitrate (Table 3). The different capacity to accumulate nitrate can correlate with a different location of the nitrate–reductase activity[16–18] as well as to a different degree of nitrate absorption and transfer in the plant.[19,20]

Nitrate content differed in the various portions under examination. Indeed, the vegetable organs analysed can be listed by decreasing nitrate content as follows: petiole > leaf > root > stem > inflorescence > tuber > bulb.

Lettuce and spinach always showed much lower nitrate contents than the maximum limits set in Regulation 194/97 of the European Commission. However, inner lettuce leaves accumulated 43% less nitrate than outer ones (Fig 1). The latter had photosynthetic efficiency lower than the former and contained larger vacuoles (nitrate accumulation sites).[21] Outer leaves can be eliminated by consumers to reduce nitrate intake. Furthermore, for spinach, where petioles and blades contributed 58 and 43% respectively to nitrate content (Fig 1), nitrate content can be reduced at harvesting by increasing the blade/petiole ratio, ie by cutting spinach higher. Over 60% of total nitrate content in Swiss chard is in the petioles (Fig 1); thus total nitrate content in the edible portion can be cut by at least 30% if half petiole is eliminated. Inner celery petioles accumulate less nitrate than outer ones (Fig 1); thus the latter can be thrown away to reduce nitrate intake.

In most of the leaf vegetables analysed, nitrate was inversely correlated with dry matter (eg Swiss chard ($p < 0.001$; $r = -0.75$), parsley ($p < 0.05$; $r = -0.81$) and spinach ($p < 0.001$; $r = -0.66$) and oxalate content (eg spinach ($p < 0.01$; $r = -0.56$) and Swiss chard ($p < 0.01$; $r = -0.52$)). Such an inverse relationship between nitrate and dry matter is associated with inefficient nitrate assimilation, which occurs for instance under conditions of low light energy. This confirms the osmotic regulatory function of NO_3 in replacement of organic acids and sugars.[21] A homeostatic relationship between nitrate uptake/reduction and organic anion accumulation has long been recognised. Malate is the dominant anion in some plants.[22] Raven and Smith[23] proposed that oxalate has the same function, with the further advantage that deposition of calcium oxalate in the vacuole will not influence the osmotic potential of the cell.

In species belonging to *Chenopodiaceae*, the high nitrate content goes together with a high oxalate content. These vegetables are particularly rich in oxalate and can be dangerous, particularly for individuals at risk of calcium oxalate stone formation in the kidneys.

The mean daily vegetable nitrate intake in the present study (approximately 71 mg) was similar to the estimated intake for Finland (71 mg),[24] the United States (66 mg)[25] and Switzerland (64 mg),[26] lower than the estimated intake of the United Kingdom (89 mg),[27] France (98 mg)[28] and the United States (86 mg),[29] but yet higher than another British estimate (41 mg)[30] and a German one (36 mg).[31] The differences in the collection of vegetable consumption data

Vegetable	Average consumption ($g\,day^{-1}$ per person)	Nitrate content ($mg\,kg^{-1}$ FM)	Nitrate mean daily intake (mg per person)	(%)
Artichoke	5.1	30[a]	0.15	0.21
Bean	1.9	18[b]	0.03	0.04
Beetroot	5.5	1727	9.50	13.41
Broadbean	1.9	40[a]	0.08	0.11
Broccoli	3.7	154	0.57	0.80
Broccoli raab	2.8	905	2.53	3.57
Cabbage	1.7	400	0.68	0.96
Carrot	4.7	195	0.92	1.30
Cauliflower	2.3	202	0.46	0.65
Celery	2.3	1678	3.86	5.45
Chicory	1.0	1452	1.45	2.05
Cucumber	2.7	79[a]	0.21	0.30
Eggplant	12.0	108[b]	1.30	1.83
Endive	3.6	224	0.81	1.14
Fennel	2.0	363	0.73	1.03
Garlic	0.7	34	0.02	0.03
Green bean	5.7	161[b]	0.92	1.30
Lettuce	17.1	832[c]	14.23	20.08
Melon	3.0	493[b]	1.48	2.09
Onion	7.3	32	0.23	0.32
Pea	3.3	40[b]	0.13	0.18
Pepper	12.1	87[b]	1.05	1.48
Potato	55.5	81	4.49	6.34
Spinach	3.1	1845	5.72	8.07
Squash	11.3	603[b]	6.81	9.61
Swiss chard	4.2	2363	9.92	14.00
Tomato	55.5	50[b]	2.77	3.91
Total			71.05	100

[a] Santamaria and Elia unpublished data.
[b] Averaged values from Refs 35 and 36.
[c] Weighted mean among the three varieties (see Table 3).

Table 4. Average daily consumption, mean nitrate (NO_3) content and mean daily nitrate intake (and related incidence) of several vegetables produced and traded in southern Italy

and in nitrate content may partly explain the discrepancies in nitrate intake between countries.

The vegetables that contributed most to daily nitrate intake were lettuce, Swiss chard and beetroot owing to their high nitrate content (Table 4). White[29] and UK MAFF[30] also agree that leaf vegetables are the major source for nitrate intake, and beetroot is the primary source (32%) of nitrate intake in Finland.[24]

Based on the hypothesis of an average vegetable consumption and that vegetables contribute 80% of total daily nitrate intake, the amount assessed in the present research (71 mg) is far below the JEFCA ADI of 219 mg for a person of 60 kg.

Vegetables, and also fruit, are rich in ascorbic acid, tocopherols, carotenoids and flavonoids, which are all compounds able to inhibit N-nitrocompound formation.[5,32,33] Furthermore, by cooking vegetables in water (with low nitrate concentration), at least 50% of accumulated nitrate is removed.[34]

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