



Nitrate in fruits and vegetables

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ABSTRACT

The current article provides an updated review of scientific advances regarding nitrate accumulation in plant tissues and a critical examination of the genetic, agroenvironmental and postharvest factors that can modulate nitrate levels in a wide range of horticultural crops, including herbs, roots and tubers, inflorescences, buds, seeds, stems, and leafy vegetables, fungi as well as fruits. A refined classification of horticultural crops is presented according to the nitrate content of their edible product. The role of plant cultivar/morphotype and tissue age in nitrate accumulation is discussed along with the physiological role of nitrate as osmoticum in maintaining turgor and driving leaf expansion under conditions of variable photosynthetic capacity. Nitrate accumulation is examined in respect to key cultural practices, such as the timing-rate-form of N application and the use of plant biostimulants (natural substances and microbial inoculants), as well as the potential interaction with other nutrients (e.g., P, Ca, Mo and Cl). The influence of environmental conditions during plant growth (light intensity, spectral quality, photoperiod, air and root-zone temperature and atmospheric CO₂ concentration), harvest stage and diurnal timing of harvest is assessed. Postharvest storage conditions (temperature, light, and duration) are discussed in respect to their effects on the putative endogenous conversion of nitrate residues to nitrites. Several approaches that may be adopted to reduce nitrate content in vegetables, fruits and herbs are analysed and warranted future research subjects are identified.

1. Introduction

Nitrate (NO₃⁻) constitutes the most important form of nitrogen (N) taken up readily in large quantities by most horticultural crops (*i.e.* vegetables, fruit trees and vines) to attain maximal yields (Baker and Mills, 1980; Colla et al., 2010, 2011). When nitrate uptake far exceeds assimilation by the plant, accumulation of nitrate in the plant tissues can occur. In non-leguminous crops, higher concentrations of nitrate tend to accumulate in the leaves while lower levels concentrate in bulbs, seeds, fruits, roots and tubers. For this reason, leafy vegetables (*i.e.*, rocket, Swiss chard, spinach, lettuce, celery and parsley) are considered as prominent nitrate-accumulating species (Maynard et al., 1976; Santamaria, 2006).

Human exposure to nitrate is mainly exogenous, deriving from the consumption of raw vegetables (80%) with minor contribution from drinking water (15%), animal products (meat and cheese) and grain (5%) (EFSA, 2008; Lundberg et al., 2008; Rathod et al., 2016). Nitrate itself is relatively harmless, since the fatal adult dose is considered to be higher than 7–35 g, which is about 100-fold higher than the acceptable

daily intake of NO₃⁻ set by the European Union (3.7 mg/kg body weight per day), equivalent to 222 mg of NO₃⁻ per day for a 60 kg individual (EU Scientific Committee for Food, 1995; Petersen and Stoltze, 1999).

Contrary to the relatively non-deleterious effect of the nitrate ion, human exposure to its reaction products and metabolites, including nitrite, nitric oxide and N-nitroso compounds, mediated by the endogenous reduction of nitrate to nitrite by salivary enzymes and oral bacteria (*e.g.* *Staphylococcus sciuri* and *Streptococcus intermedius*), may pose direct threat to human health (EFSA, 2008). Health concerns over nitrite presence in the human body originate from its association with gastric and bladder cancers, and also from nitrite implication in the methaemoglobinaemia syndrome, colloquially referred to as ‘blue baby syndrome’ (Abdel Mohsen et al., 1999; Mensinga et al., 2003; Parks et al., 2008). However, the significance of nitrate intake on human health is still uncertain, since several clinical and pre-clinical studies did not confirm any correlation between dietary nitrate and carcinogenesis (Milkowski et al., 2010; Nie et al., 2009). Underlying causes for the absence of such correlation might be the simultaneous intake of

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Table 1

Maximum nitrate content for the commercialization of fresh vegetables according to Commission regulation (EU) No 1258/2011.

Vegetable	Harvest period and/or growing conditions	Maximum levels (mg NO ₃ kg ⁻¹ fw)
Spinach (<i>Spinacia oleracea</i> L.)	–	3,500
Lettuce (<i>Lactuca sativa</i> L.) (protected and open-grown lettuce) excluding 'Iceberg' type lettuce	1st October to 31st March (lettuce grown under cover)	5000
	1st October to 31st March (lettuce grown in the open air)	4,000
	1st April to 30th September (lettuce grown under cover)	4,000
	1st April to 30th September (lettuce grown in the open air)	3000
'Iceberg' type lettuce	Lettuce grown under cover	2,500
	Lettuce grown in the open air	2,000
Rucola (<i>Eruca sativa</i> Mill., <i>Diplotaxis</i> sp., <i>Brassica tenuifolia</i> Baill., <i>Sisymbrium tenuifolium</i> L.)	1st October to 31st March	7,000
	1st April to 30th September	6,000

vitamin C from the same food sources and the washing away of soluble nitrates during food preparation such as boiling of vegetables. Despite the ongoing debate on the putative beneficial or harmful effects of nitrate compounds on human health, the production and wide commercialization of nitrate-rich leafy vegetables and processed cereal-based foods is subject to strict regulatory limitations (Cavauiolo and Ferrante, 2014), since some target population groups (vegetarians, infants and elderly) could be at higher risk of developing cancer, when exposed to excessive dietary intakes of nitrate (Cavauiolo and Ferrante, 2014; EFSA, 2008). As a precautionary measure, the European Commission regulations N° 1881/2006 and 1258/2011 established nitrate thresholds for the following six food crops: fresh spinach (–3500 mg/kg fresh weight; FW); preserved, deep frozen or frozen spinach (2000 mg/kg FW); fresh lettuce (3000–5000 mg/kg FW); Iceberg type lettuce (2000–2500 mg/kg FW); salad and wild rocket [*Eruca sativa* Mill., *Diplotaxis tenuifolia* (L.) DC., *Brassica tenuifolia* (L.) Baill., *Sisymbrium tenuifolium* L. (6000–7000 mg/kg FW)]; processed cereal-based foods and baby foods for infants and young children (200 mg/kg FW) (Table 1). Because of the different environmental conditions, cultivation systems (open field or protected cultivation) and eating habits in EU countries, the threshold values of nitrates for the three leafy vegetables vary among species and growing season (April–September: period of high light intensity and duration; October–March: period of low light intensity and duration; Table 1).

The accumulation of nitrates in raw vegetables, herbs and fruits depends upon many preharvest factors such as plant species/genotype, agronomic factors (e.g. timing, concentration and form of N application), prevailing environmental conditions during plant growth (e.g. light intensity, spectral quality, photoperiod, air temperature and carbon dioxide concentration), harvest stage, as well as the time of harvest during the day (Andrews et al., 2013; Blom-Zandstra and Eenink, 1986; Chadja et al., 1999; Colonna et al., 2016; Demisar and Osvold, 2003; Escobar-Gutierrez et al., 2002; Fallovo et al., 2009; Gaudreau et al., 1995; McCall and Willumsen, 1998; Scaife et al., 1986; Siomos, 2000; Tesi and Lenzi, 1998). Moreover, the postharvest factors in particular the storage conditions might also elicit or inhibit the endogenous conversion of nitrates to nitrites (Riens and Heldt, 1992). Many studies have demonstrated that the genetic background, nitrate supply and light conditions are the three predominant factors affecting plant nitrate levels (Amr and Hadidi, 2001; Elia et al., 1998; Santamaria et al., 2001). For these reasons, in recent years, growers, extension specialists and scientists are seeking different strategies for reducing anti-nutritional compounds like nitrates, most notably present in leafy vegetables. The various approaches that may be adopted to reduce the nitrate content in plants include: 1) the reduction of nitrate concentration in the nutrient solution when fertigation is applied (Marsic and Osvold, 2002a, 2002b); 2) the partial replacement of nitrate-based fertilizers with other N forms (ammonium and urea) (Borgognone et al., 2013); 3) a short nitrate-starvation obtained by replacing the nutrient

solution with a nitrate-free one or with fresh water for one to five days before harvest (Borgognone et al., 2016); 4) replacement of nitrates (e.g., calcium nitrate) with chlorides (e.g., calcium chloride) (Borgognone et al., 2016); 5) modulation of production environment, including light spectral composition (Blom-Zandstra and Lampe, 1985; Gaudreau et al., 1995); and 6) use of genotypes with low nitrate accumulation capacity (Burns et al., 2011; Escobar-Gutierrez et al., 2002).

The issue of nitrates in vegetables, encompassing the aspects of toxicity, content, intake and EC regulation, has been previously reviewed by Santamaria (2006). The current article aims at providing an updated review of scientific advances on nitrate accumulation in plant tissues and a critical examination of the genetic, agronomic and environmental factors that can modulate nitrate levels in a wide range of horticultural crops, including herbs, roots and tubers, inflorescences, buds, seed, stem, fruits and leafy vegetables, as well as fungi. Additionally, the postharvest fate of nitrate and nitrite in fruits and vegetables is critically examined. The review concludes by identifying several approaches that may be adopted to reduce nitrate content in vegetables, fruits and herbs.

2. Nitrate accumulation in crops and plant tissues

A large variation in nitrate accumulation has been reported among plant species and even among cultivars of the same species (Blom-Zandstra, 1989; Blom-Zandstra and Eenink, 1986; Maynard et al., 1976; Ostrem and Collins, 1983; Quinche and Dvorak, 1980; Reinink and Eenink, 1988; Rouphael et al., 2017a), while the magnitude of this variation is often modulated by environmental conditions (Table 2). Variation among plant species concerning nitrate content was also related to the edible plant parts in each crop (Anjana et al., 2006; Santamaria et al., 1999). Because nitrate is transported through the xylem by the transpiration stream (Pate, 1980), leafy vegetables bearing large laminae tend to accumulate higher nitrates, especially in the vacuoles of mesophyll cells, compared to other types of vegetables that produce fruits or hypogean storage organs.

Maynard et al. (1976) reported that nitrates are lowest in floral parts and increasing concentrations were found in fruit or grain, leaves, roots, and petioles or stems. It has been demonstrated that nitrate concentration in the petiole is more than twice as high as in the lamina of rocket leaf (Elia et al., 2000) and the difference could be as high as 6.6-fold in the case of spinach (Anjana et al., 2006). Nitrate concentration in the petioles + plus stems was also higher than in the leaves, whereas the lowest values were recorded in the roots of rape, cabbage and spinach (Chen et al., 2004). Moreover, nitrate content of asparagus chicory stems was lower than in leaves (Santamaria et al., 1999). In spinach, the leaf laminae accumulate less nitrate than the petioles (Santamaria et al., 1999), and increasing the lamina/petiole ratio by trimming petioles at harvest (Santamaria et al., 1999) reduces the overall nitrate content of the product. In lettuce, not only higher

Table 2
Nitrate content in vegetables, fruits, herbs, and mushrooms.

Common name	Scientific name	Production system	Season	Nitrate (mg/kg FW)		Reference
				Mean	Min-max	
Brassica vegetables						
Bok choy	<i>Brassica rapa</i> L. subsp. <i>chinensis</i> L.	Conventional	Summer	1933	(729–3430)	Zhou et al. (2000)
		Conventional	Summer	1416	(664–2131)	Zhou et al. (2000)
Broccoli	<i>Brassica oleracea</i> L. var. <i>italica</i> Plenck.	Conventional	Summer	130		Zhou et al. (2000)
		Conventional	Aug–Jun	70	(30–85)	Lyons et al. (1994)
		Organic	Dec	ND		Lyons et al. (1994)
		Conventional	Apr, Aug–Sep	154	(ND–440)	Santamaria et al. (1999)
		Conventional	Unspecified	279	(16–758)	EFSA (2008)
		Conventional	Unspecified	395		Hord et al. (2009)
		Conventional	Sep–Nov, Feb–Mar	394	(29–1140)	Núñez de González et al. (2015)
		Organic	Sep–Nov, Feb–Mar	204	(3–683)	Núñez de González et al. (2015)
Broccoli raab	<i>Brassica rapa</i> L.	Conventional	Jan–Jul	905	(321–1705)	Santamaria et al. (1999)
Brussels sprouts	<i>Brassica oleracea</i> L. var. <i>gemmifera</i> Zenker	Conventional	Unspecified	6	(0–38)	Greenwood et al. (1986)
		Conventional	Aug–Jun	10	(10–15)	Lyons et al. (1994)
Cabbage	<i>Brassica oleracea</i> L. var. <i>capitata</i> L.	Conventional	Unspecified	24	(1–100)	EFSA (2008)
		Conventional	Unspecified	784		Siciliano et al. (1975)
		Conventional	Unspecified	165		Maynard et al. (1976)
		Conventional	Winter	116	(9–334)	Greenwood et al. (1986)
		Conventional	Summer	74	(0–343)	Greenwood et al. (1986)
		Conventional	Aug–Jun	240	(70–370)	Lyons et al. (1994)
		Conventional	Fall	412		Penttilä (1995)
		Conventional	Summer	812		Penttilä (1995)
		Conventional	Aug	400	(8–929)	Santamaria et al. (1999)
		Conventional	Winter	94	(26–268)	Ysart et al. (1999)
		Conventional	Summer	488	(37–1523)	Ysart et al. (1999)
		Conventional	Unspecified	209	(20–414)	Fytianos and Zarogiannis (1999)
		Conventional	Summer	1558	(963–2854)	Zhou et al. (2000)
		Conventional	Summer	1360		Zhou et al. (2000)
Cauliflower	<i>Brassica oleracea</i> L. var. <i>botrytis</i> L.	Conventional	Summer	479	(173–734)	Zhou et al. (2000)
		Organic	Unspecified	197		Yordanov et al. (2001)
		Conventional	Unspecified	1530	(26–2670)	Zhong et al. (2002)
		Conventional	Nov–Mar	730	(29–1498)	Chung et al. (2003)
		Conventional	Apr–Oct	722	(1–1788)	Chung et al. (2003)
		Conventional	Unspecified	437	(74–1138)	Tamme et al. (2006)
		Conventional	May–Oct	881	(112–1864)	Susin et al. (2006)
		Conventional	Unspecified	311	(47–833)	EFSA (2008)
		Organic	Unspecified	102	± 171 (SD)	González et al. (2010)
		Conventional	Aug–Jun	50	(40–75)	Lyons et al. (1994)
		Conventional	Winter	57	(54–59)	Ysart et al. (1999)
		Conventional	Summer	87	(73–102)	Ysart et al. (1999)
		Conventional	Aug	202	(143–354)	Santamaria et al. (1999)
		Conventional	Unspecified	420	(40–1030)	Souci et al. (1999)
		Conventional	Summer	497	(448–556)	Zhou et al. (2000)
		Conventional	Unspecified	287	(104–404)	Tamme et al. (2006)
Conventional	Unspecified	148	(7–390)	EFSA (2008)		
Chinese cabbage	<i>Brassica rapa</i> L.	Organic	Unspecified	38	± 36 (SD)	González et al. (2010)
		Conventional	Fall	1081		Penttilä (1995)
		Conventional	Summer	964		Penttilä (1995)
		Conventional	Summer	1300	(429–1610)	Zhou et al. (2000)
		Conventional	Unspecified	2120	(337–3600)	Zhong et al. (2002)
		Conventional	Winter	1291	(131–3249)	Chung et al. (2003)
		Conventional	Summer	2009	(208–5490)	Chung et al. (2003)
		Conventional	Unspecified	1243	(232–2236)	Tamme et al. (2006)
		Conventional	Unspecified	933	(77–1928)	EFSA (2008)
		Conventional	Unspecified	885	(212–1844)	Tamme et al. (2010)
		Conventional	Unspecified	1344	(340–2236)	Tamme et al. (2010)
		Conventional	Sep–Nov, Feb–Mar	418	(37–1831)	Núñez de González et al. (2015)
		Organic	Sep–Nov, Feb–Mar	552	(2–2114)	Núñez de González et al. (2015)
		Curly Kale	<i>Brassica oleracea</i> L.	Conventional	Unspecified	537
Kohlrabi	<i>Brassica oleracea</i> L. var. <i>gongyloides</i> L.	Conventional	May, Aug	1769	(1551–2046)	Santamaria et al. (1999)
		Conventional	Unspecified	987	(142–1830)	EFSA (2008)
Mustard greens	<i>Brassica juncea</i> (L.) Czern.	Conventional	Summer	1852	(723–2838)	Zhou et al. (2000)
		Conventional	Summer	2987	(2140–3897)	Zhou et al. (2000)
		Conventional	Summer	1790		Zhou et al. (2000)
		Conventional	Unspecified	1160		Hord et al. (2009)
Red cabbage	<i>Brassica oleracea</i> L.	Conventional	Unspecified	281	(35–704)	EFSA (2008)
Savoy cabbage	<i>Brassica oleracea</i> L. var. <i>sabauda</i> L.	Conventional	Apr–Jun, Sep	29	(26–32)	Santamaria et al. (1999)

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Table 2 (continued)

Common name	Scientific name	Production system	Season	Nitrate (mg/kg FW)		Reference
				Mean	Min-max	
Swiss chard	<i>Brassica vulgaris</i> L. var <i>cicla</i> L.	Conventional	Unspecified	324	(1–1144)	EFSA (2008)
		Conventional	Aug–Jun	570	(450–725)	Lyons et al. (1994)
		Conventional	Jan–Jul	2363	(1299–4220)	Santamaria et al. (1999)
		Conventional	Unspecified	1061		Herencia et al. (2007)
		Organic	Unspecified	749		Herencia et al. (2007)
		Conventional	Unspecified	1690	(178–3685)	EFSA (2008)
		Conventional	Unspecified	3003	(53–4774)	Parks et al. (2008)
		Organic	Unspecified	2630	± 1860 (SD)	González et al. (2010)
baby salads		Conventional	Unspecified	23	± 7 (SE)	Aires et al. (2013)
Leafy vegetables						
Amaranth	<i>Amaranthusspp.</i>	Conventional	Summer	1180		Zhou et al. (2000)
Arugula (rocket)	<i>Eruca sativa</i> L.	Conventional	Unspecified	2167	(439–3483)	EFSA (2008)
		Conventional	Unspecified	1399	(691–2626)	Jana and Muktan (2013)
		Conventional	Unspecified	4677	(1528–7340)	EFSA (2008)
		Conventional	Jun–Feb	5377	(3726–8268)	Guadagnin et al. (2005)
	<i>Eruca vesicaria</i> L.	Hydroponic	Jun–Feb	8243	(6461–9703)	Guadagnin et al. (2005)
		Organic	Jun–Feb	4073	(2160–5670)	Guadagnin et al. (2005)
		Conventional	Jan–Jul	2597	(963–4305)	Santamaria et al. (1999)
		Conventional	Unspecified	8150	(7300–9000)	Tamme et al. (2010)
Baby salads		Conventional	Unspecified	3488		Temme et al. (2011)
Beet	<i>Beta vulgaris</i> L. subsp. <i>vulgaris</i> L.	Conventional	Unspecified	18	± 4 (SE)	Aires et al. (2013)
Belgian endive	<i>Cichorium intybus</i> L.	Conventional	Unspecified	1852	(84–3685)	EFSA (2008)
Chicory (radicchio)	<i>Cichorium intybus</i> L.	Conventional	Unspecified	1465	(63–3063)	EFSA (2008)
Dandelion	<i>Taraxacum officinale</i> F.H.Wigg.	Conventional	Unspecified	355	(5–829)	EFSA (2008)
Endive (escarole)	<i>Cichorium endivia</i> L.	Conventional	Unspecified	77		Temme et al. (2011)
		Conventional	Unspecified	605	(5–2747)	EFSA (2008)
		Conventional	Unspecified	663		Siciliano et al. (1975)
		Conventional	Apr, Aug–Sep	224	(51–698)	Santamaria et al. (1999)
Fennel	<i>Foeniculum vulgare</i> Mill. subsp. <i>vulgare</i> var <i>azoricum</i> (Mill.) Thell.	Conventional	Unspecified	523	(6–1579)	EFSA (2008)
		Conventional	Unspecified	1414	(washed –11.4%)	Temme et al. (2011)
		Conventional	Jan–Sep	363	(107–769)	Santamaria et al. (1999)
Green onion (scallion)	<i>Allium cepa</i> L.	Conventional	Jan–Jul	410	(69–1046)	Santamaria et al. (1999)
		Conventional	Summer	314	(262–367)	Zhou et al. (2000)
		Conventional	Unspecified	704	(8–4240)	Zhong et al. (2002)
		Conventional	Nov–Mar	392	(10–1364)	Chung et al. (2003)
		Conventional	Apr–Oct	463	(4–1676)	Chung et al. (2003)
		Conventional	Unspecified	477	(160–920)	Tamme et al. (2006)
		Conventional	Unspecified	533	(99–870)	Tamme et al. (2010)
		Conventional	Jan–Jul	1452	(446–2284)	Santamaria et al. (1999)
		Conventional	Unspecified	170		Maynard et al. (1976)
		Conventional	Unspecified	425	(33–2304)	Greenwood et al. (1986)
		Conventional	Aug–Jun	213	(65–330)	Lyons et al. (1994)
		Lettuce	<i>Lactuca sativa</i> L.	Conventional	Winter	3205
Conventional	Summer			2219		Dejonckheere et al. (1994)
Conventional	Fall			2443		Penttilä (1995)
Conventional	Summer			1050		Penttilä (1995)
Conventional	Winter			887	(482–1435)	Ysart et al. (1999)
Conventional	Summer			1568	(610–3857)	Ysart et al. (1999)
Conventional	Unspecified			282	(8–808)	Fytianos and Zarogiannis (1999)
Conventional	Summer			896	(580–1454)	Zhou et al. (2000)
Conventional	Summer			610		Zhou et al. (2000)
Conventional	Nov–Mar			1933	(247–3283)	Chung et al. (2003)
Conventional	Apr–Oct			2728	(884–4488)	Chung et al. (2003)
Conventional	Unspecified			2167	(397–3230)	Tamme et al. (2006)
Conventional	May–Oct			1074	(21–3986)	Susin et al. (2006)
Conventional	Jun–Feb			1303	(677–2179)	Guadagnin et al. (2005)
Hydroponic	Jun–Feb			2983	(1842–4022)	Guadagnin et al. (2005)
Organic	Jun–Feb	818	(115–1852)	Guadagnin et al. (2005)		
Conventional	Winter	3325	(2156–4795)	Tamme et al. (2010)		
Conventional	Summer	2720	(1410–4000)	Tamme et al. (2010)		
Baby salads (green)	Conventional	Unspecified	26	± 2 (SE)	Aires et al. (2013)	
	Organic	Unspecified	6	± 1 (SE)	Aires et al. (2013)	
Baby salads (red)	Conventional	Unspecified	45	± 5 (SE)	Aires et al. (2013)	
	Organic	Unspecified	5	± 2 (SE)	Aires et al. (2013)	
Butterhead		Conventional	Jan–Jul	1089	(672–1745)	Santamaria et al. (1999)
Iceberg		Conventional	Unspecified	1100, 1300		Siciliano et al. (1975)
		Conventional	Jan–Jul	581	(428–810)	Santamaria et al. (1999)
		Conventional	Unspecified	875	(210–1537)	EFSA (2008)
		Conventional	Winter	970	(870–1100)	Muramoto (1999)

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Table 2 (continued)

Common name	Scientific name	Production system	Season	Nitrate (mg/kg FW)		Reference	
				Mean	Min-max		
Romaine (cos)		Conventional	Summer	707	(520–1100)	Muramoto (1999)	
		Organic	Winter	977	(760–1300)	Muramoto (1999)	
		Organic	Summer	575	(480–660)	Muramoto (1999)	
		Conventional	Unspecified	1084		Temme et al. (2011)	
		Conventional	Unspecified	1400		Siciliano et al. (1975)	
		Conventional	Jan–Jul	1241	(684–1766)	Santamaria et al. (1999)	
		Conventional	Sep–Nov, Feb–Mar	851	(79–2171)	Núñez de González et al. (2015)	
		Organic	Sep–Nov, Feb–Mar	844	(58–2013)	Núñez de González et al. (2015)	
		Conventional	Winter	1030	(890–1200)	Muramoto (1999)	
		Conventional	Summer	1140	(770–1700)	Muramoto (1999)	
Salad mix		Organic	Winter	1170	(820–1500)	Muramoto (1999)	
		Organic	Summer	954	(580–1600)	Muramoto (1999)	
		Conventional	Unspecified	1105	(167–2200)	EFSA (2008)	
		Conventional	Dec–Jan	819		Siciliano et al. (1975)	
		Conventional	Unspecified	821		Hord et al. (2009)	
Sea beet	<i>Beta vulgaris</i> L. subsp. <i>maritima</i> (L.) Arcang.	Organic	Unspecified	2595	± 1228 (SD)	González et al. (2010)	
Spinach		<i>Spinacia oleracea</i> L.	Conventional	Dec–Jan	2220	± 375 (SD)	Siciliano et al. (1975)
		Conventional	Unspecified	524		Maynard et al. (1976)	
		Conventional	Unspecified	284	(8–1513)	Greenwood et al. (1986)	
		–	Winter	2548		Dejonckheere et al. (1994)	
		–	Summer	2108		Dejonckheere et al. (1994)	
		Conventional	Winter	1272	(839–1721)	Ysart et al. (1999)	
		Conventional	Summer	1639	(1009–2194)	Ysart et al. (1999)	
		Conventional	Winter	2230	(1500–2900)	Muramoto (1999)	
		Conventional	Summer	2850	(2000–3400)	Muramoto (1999)	
	Organic	Winter	1800	(890–2600)	Muramoto (1999)		
	Organic	Summer	1820	(600–3000)	Muramoto (1999)		
	Conventional	Jan–Jul	1845	(547–3350)	Santamaria et al. (1999)		
	Conventional	Unspecified	1250	(545–3760)	Fytianos and Zarogiannis (1999)		
	Conventional	Summer	2538	(239–3872)	Zhou et al. (2000)		
	Conventional	Summer	1850		Zhou et al. (2000)		
	Conventional	Summer	1649	(1012–2356)	Zhou et al. (2000)		
	Conventional	Nov–Mar	3334	(427–7439)	Chung et al. (2003)		
	Conventional	Apr–Oct	4814	(195–7793)	Chung et al. (2003)		
	Conventional	Jun	1760	± 440 (SD)	Jaworska (2005)		
	Conventional	Unspecified	2508	(2508)	Tamme et al. (2006)		
	Conventional	Unspecified	1066	(64–3048)	EFSA (2008)		
	Conventional	Unspecified	7410		Hord et al. (2009)		
	Conventional	Winter	2584	(2508–2660)	Tamme et al. (2010)		
	Conventional	Summer	2090	(340–3650)	Tamme et al. (2010)		
	Organic	Unspecified	1170	± 967 (SD)	González et al. (2010)		
	Conventional	Sep–Nov, Feb–Mar	2797	(65–8000)	Núñez de González et al. (2015)		
	Organic	Sep–Nov, Feb–Mar	1318	(16–4089)	Núñez de González et al. (2015)		
Watercress	<i>Nasturtium officinale</i> R. Br.	Conventional	Unspecified	136	(4–174)	EFSA (2008)	
		Conventional	Jun–Feb	1234	(296–2388)	Guadagnin et al. (2005)	
		Hydroponic	Jun–Feb	4873	(2009–6160)	Guadagnin et al. (2005)	
		Organic	Jun–Feb	5180	(3340–5926)	Guadagnin et al. (2005)	
Baby salads		Conventional	Unspecified	43	± 7.2 (SE)	Aires et al. (2013)	
		Organic	Unspecified	1.5	± 0.3 (SE)	Aires et al. (2013)	
Herbs			Unspecified				
Basil	<i>Ocimum basilicum</i> L.	Conventional	Unspecified	2292	(94–5174)	EFSA (2008)	
		Conventional	Unspecified	4695	(4040–5350)	Tamme et al. (2010)	
Borage	<i>Borago officinalis</i> L.	Conventional	Unspecified	1918	(200–4550)	EFSA (2008)	
Chives	<i>Allium tuberosum</i> L.	Conventional	Nov–Mar	1020	(863–9323)	Chung et al. (2003)	
		Conventional	Apr–Oct	5828	(3016–9638)	Chung et al. (2003)	
		Conventional	Unspecified	748	(1–2949)	EFSA (2008)	
Coriander	<i>Coriandrum sativum</i> L.	Conventional	Summer	380		Zhou et al. (2000)	
		Conventional	Unspecified	2445	(1135–3982)	EFSA (2008)	
Dill	<i>Anethum graveolens</i> L.	Conventional	Unspecified	2936	(2236–3267)	Tamme et al. (2006)	
		Conventional	Unspecified	1332	(13–4294)	EFSA (2008)	
		Conventional	Unspecified	3911	(2670–5290)	Tamme et al. (2010)	
Parsley	<i>Petroselinum crispum</i> (Mill.) Fuss.	Conventional	Aug–Jun	220	(10–330)	Lyons et al. (1994)	
		–	Winter	2813		Dejonckheere et al. (1994)	
		–	Summer	2488		Dejonckheere et al. (1994)	

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Table 2 (continued)

Common name	Scientific name	Production system	Season	Nitrate (mg/kg FW)		Reference
				Mean	Min-max	
		Conventional	Mar–Sep	1150	(366–1851)	Santamaria et al. (1999)
		Conventional	Unspecified	966	(674–1588)	Tamme et al. (2006)
		Conventional	Unspecified	958	(10–3404)	EFSA (2008)
		Conventional /Under cover	Unspecified	2134	(887–3590)	Tamme et al. (2010)
Thyme	<i>Thymus</i> spp.	Conventional /Under cover	Unspecified	4290	(4290)	Tamme et al. (2010)
Fruit vegetables						
Cucumber	<i>Cucumis sativas</i> L.	Conventional	Unspecified	24		Siciliano et al. (1975)
		–	Winter	322		Dejonckheere et al. (1994)
		–	Summer	363		Dejonckheere et al. (1994)
		Conventional	Unspecified	157	(20–576)	Fytianos and Zarogiannis (1999)
		Conventional	Summer	65	(12–143)	Zhou et al. (2000)
		Conventional	Summer	485	(227–691)	Zhou et al. (2000)
		Conventional	Summer	104	(82–160)	Zhou et al. (2000)
		Conventional	Unspecified	59		Yordanov et al. (2001)
		Conventional	Unspecified	170	(17–500)	Zhong et al. (2002)
		Conventional	Nov–Mar	267	(83–580)	Chung et al. (2003)
		Conventional	Apr–Oct	180	(1–649)	Chung et al. (2003)
		Conventional	May–Oct	93	(4–245)	Susin et al. (2006)
		Conventional	Unspecified	160	(< 30–1236)	Tamme et al. (2006)
		Conventional	Unspecified	185	(22–409)	EFSA (2008)
		Conventional	Unspecified	335	(89–740)	Tamme et al. (2010)
		Conventional	Unspecified	240	(124–372)	Tamme et al. (2010)
Eggplant (aubergine)	<i>Solanum melongena</i> L.	Conventional	Unspecified	302		Siciliano et al. (1975)
		Conventional	Summer	308	(250–424)	Zhou et al. (2000)
		Conventional	Summer	58		Zhou et al. (2000)
		Conventional	Summer	440	(125–1225)	Zhou et al. (2000)
		Conventional	Unspecified	479	(67–1000)	Zhong et al. (2002)
		Conventional	Unspecified	314	(29–572)	EFSA (2008)
		Organic	Unspecified	90	(24–135)	González et al. (2010)
		Conventional	Unspecified	302		Temme et al. (2011)
Gherkin	<i>Cucumis anguria</i> L.	Conventional	Unspecified	69	(11–230)	EFSA (2008)
Towel gourd	<i>Luffa</i> spp.	Conventional	Summer	514	(514)	Zhou et al. (2000)
		Conventional	Summer	45		Zhou et al. (2000)
Wax gourd	<i>Benincasa hispida</i> Cogn.	Conventional	Summer	541	(358–680)	Zhou et al. (2000)
		Conventional	Summer	495	(187–750)	Zhou et al. (2000)
		Conventional	Summer	52	(4–254)	Zhou et al. (2000)
		Conventional	Summer	40		Zhou et al. (2000)
		Conventional	Unspecified	635	(201–1390)	Zhong et al. (2002)
Marrow	<i>Cucurbita pepo</i> L.	Conventional	Unspecified	884		Herencia et al. (2007)
		Conventional	Unspecified	222		Herencia et al. (2007)
		Organic	Unspecified	405		Herencia et al. (2007)
		Organic	Unspecified	250		Herencia et al. (2007)
		Organic	Unspecified	43	± 26 (SD)	González et al. (2010)
Melon	<i>Cucumis melo</i> L.	Conventional	Unspecified	221	(peeled–41.1%)	Temme et al. (2011)
		Conventional	May–Aug	48	(41–56)	Colla et al. (2010)
Pepper	<i>Capsicum</i> L.	Conventional	Aug–Jun	20	(20–45)	Lyons et al. (1994)
		Hydroponic	Dec	15		Lyons et al. (1994)
		Conventional	Unspecified	82		Herencia et al. (2007)
		Organic	Unspecified	18		Herencia et al. (2007)
		Conventional	Unspecified	108	(1–476)	EFSA (2008)
Chili pepper	<i>Capsicum</i> L.	Conventional	Unspecified	234	(135–382)	Zhou et al. (2000)
		Conventional	Unspecified	67	(4–120)	EFSA (2008)
Green pepper	<i>Capsicum annuum</i> L.	Conventional	Unspecified	62		Siciliano et al. (1975)
		Conventional	Summer	142	(30–267)	Zhou et al. (2000)
		Conventional	Summer	65		Zhou et al. (2000)
		Conventional	Summer	276	(196–396)	Zhou et al. (2000)
		Conventional	Nov–Mar	95	(2–559)	Chung et al. (2003)
		Conventional	Apr–Oct	65	(1–225)	Chung et al. (2003)
		Conventional	Unspecified	93		Temme et al. (2011)
Pumpkin	<i>Cucurbita pepo</i> L. var <i>pepo</i> L.H. Bailey	Conventional	Aug–Jun	392	(145–790)	Lyons et al. (1994)
		Conventional	Unspecified	424	(205–748)	Zhou et al. (2000)
		Conventional	Unspecified	240		Zhou et al. (2000)
		Conventional	Unspecified	252	(110–852)	Zhou et al. (2000)
		Conventional	Unspecified	894	(8–4617)	EFSA (2008)
		Conventional	Unspecified	174	(< 30–445)	Tamme et al. (2006)
Strawberry	<i>Fragaria</i> × <i>ananassa</i> Duch.	Conventional	Unspecified	18		Yordanov et al. (2001)
		Conventional	Unspecified	55	(< 30–111)	Tamme et al. (2006)
		Conventional	May–Oct	94	(9–360)	Susin et al. (2006)

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Table 2 (continued)

Common name	Scientific name	Production system	Season	Nitrate (mg/kg FW)		Reference
				Mean	Min-max	
Squash		Conventional	Unspecified	156		Temme et al. (2011)
Acorn	<i>Cucurbita pepo</i> L. var <i>turbinata</i> Paris	Conventional	Unspecified	34		Siciliano et al. (1975)
Butternut	<i>Cucurbita moschata</i> L.	Conventional	Unspecified	678		Siciliano et al. (1975)
Zucchini (courgette)	<i>Cucurbita pepo</i> L. var <i>cylindrica</i> Paris	Conventional	Unspecified	665		Siciliano et al. (1975)
		Conventional	Unspecified	27		Yordanov et al. (2001)
		Conventional	Unspecified	421	(330–511)	Tamme et al. (2006)
		Conventional	Unspecified	416	(11–1060)	EFSA (2008)
		Conventional	Unspecified	897		Temme et al. (2011)
Tomato	<i>Solanum lycopersicum</i> L.	Conventional	Unspecified	20		Maynard et al. (1976)
		Conventional	Aug–Jun	10	(ND–20)	Lyons et al. (1994)
		Conventional	Unspecified	34	(8–55)	Fytianos and Zarogiannis (1999)
		Conventional	Unspecified	17	(4–42)	Ysart et al. (1999)
		Conventional	Summer	27	(1–131)	Zhou et al. (2000)
		Conventional	Summer	238	(190–347)	Zhou et al. (2000)
		Conventional	Summer	76		Zhou et al. (2000)
		Conventional	Summer	16	(6–19)	Zhou et al. (2000)
		Conventional	Unspecified	78	(10–259)	Zhong et al. (2002)
		Conventional	May–Oct	4	(2–12)	Susin et al. (2006)
		Conventional	Unspecified	41	(< 30–100)	Tamme et al. (2006)
		Conventional	Unspecified	15		Herencia et al. (2007)
		Organic	Unspecified	5		Herencia et al. (2007)
		Conventional	Unspecified	43	(1–144)	EFSA (2008)
		Conventional	Unspecified	392		Hord et al. (2009)
		Conventional	Unspecified	36		Temme et al. (2011)
Watermelon	<i>Citrullus lanatus</i> var. <i>lanatus</i> (Thunb.) Matsum. & Nakai	Conventional	May–Aug	10		Proietti et al. (2008)
		Conventional	Summer	12	(7–18)	Zhou et al. (2000)
		Conventional	Summer	33		Zhou et al. (2000)
		Conventional	Unspecified	95	(95)	Tamme et al. (2006)
Legumes						
Bean	<i>Phaseolus vulgaris</i> L.	Conventional	Aug–Jun	60	(30–140)	Lyons et al. (1994)
		Conventional	Unspecified	186		Herencia et al. (2007)
		Organic	Unspecified	151		Herencia et al. (2007)
		Conventional	Unspecified	392	(6–810)	EFSA (2008)
		Conventional	Unspecified	585		Temme et al. (2011)
French bean		Conventional	Unspecified	756	(4–3970)	EFSA (2008)
Green bean		Conventional	Unspecified	110	(10–190)	Greenwood et al. (1986)
		Conventional	Unspecified	323	(9–735)	EFSA (2008)
		Conventional	Unspecified	26		Maynard et al. (1976)
Kidney bean		Conventional	Summer	412	(412)	Zhou et al. (2000)
		Conventional	Summer	1145	(1039–1252)	Zhou et al. (2000)
		Conventional	Summer	120		Zhou et al. (2000)
		Conventional	Summer	535	(194–1030)	Zhou et al. (2000)
String bean		Conventional	May–Oct	298	(82–675)	Susin et al. (2006)
		Conventional	Unspecified	618	(170–900)	EFSA (2008)
Pea	<i>Pisum sativum</i> L.	Conventional	Unspecified	26		Maynard et al. (1976)
		Conventional	Unspecified	1	(0–3)	Greenwood et al. (1986)
		Conventional	Aug–Jun	15	(ND–20)	Lyons et al. (1994)
		Conventional	Unspecified	150		Zhou et al. (2000)
		Conventional	Unspecified	30	(1–100)	EFSA (2008)
		Conventional	Unspecified	15		Temme et al. (2011)
Soybean sprout	<i>Glycine max</i> (L.) Merr.	Conventional	Nov–Mar	63	(15–193)	Chung et al. (2003)
		Conventional	Apr–Oct	52	(2–158)	Chung et al. (2003)
Stem vegetables						
Asparagus	<i>Asparagus officinalis</i> L.	Conventional	Unspecified	25		Maynard et al. (1976)
		Conventional	Unspecified	209	(1–1459)	EFSA (2008)
Asparagus chicory	<i>Cichorium intybus</i> L.	Conventional	Jan–Jul	498	(167–889)	Santamaria et al. (1999)
Celery	<i>Apium graveolens</i> L. var <i>dulce</i> (Mill.) Pers.	Conventional	Unspecified	1600, 2390, 2670		Siciliano et al. (1975)
		Conventional	Unspecified	535		Maynard et al. (1976)
		Conventional	Aug–Jun	295	(160–1075)	Lyons et al. (1994)
		Conventional	Jan–Jul	1678	(1009–2163)	Santamaria et al. (1999)
		Conventional	Unspecified	250	(43–692)	Fytianos and Zarogiannis (1999)
		Conventional	Summer	908	(119–1589)	Zhou et al. (2000)
		Conventional	Summer	2045	(1287–3169)	Zhou et al. (2000)
		Conventional	Summer	2090		Zhou et al. (2000)
		Conventional	Unspecified	3600	(446–10,800)	Zhong et al. (2002)
		Conventional	Unspecified	565	(256–830)	Tamme et al. (2006)
		Conventional	Unspecified	1103	(18–3319)	EFSA (2008)
		Conventional	Unspecified	660	(256–1113)	Tamme et al. (2010)

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Table 2 (continued)

Common name	Scientific name	Production system	Season	Nitrate (mg/kg FW)		Reference		
				Mean	Min-max			
Fennel	<i>Foeniculum vulgare</i> Mill.	Conventional	Unspecified	2110		Temme et al. (2011)		
		Conventional	Sep–Nov, Feb–Mar	1496	(20–4269)	Nuñez de González et al. (2015)		
		Organic	Sep–Nov, Feb–Mar	912	(1–3589)	Nuñez de González et al. (2015)		
		Conventional	Summer	1787	(1062–2219)	Zhou et al. (2000)		
		Conventional	Unspecified	1024	(25–3047)	EFSA (2008)		
		Conventional	Summer	35		Zhou et al. (2000)		
Garlic bolt	<i>Allium sativum</i> L.	Conventional	Summer	1198	(1095–1257)	Zhou et al. (2000)		
Leek	<i>Allium ampeloprasum</i> L.	Conventional	Unspecified	158	(23–1012)	Greenwood et al. (1986)		
		Conventional	Unspecified	132	(43–333)	Fytianos and Zarogiannis (1999)		
Rhubarb		Conventional	Summer	53		Zhou et al. (2000)		
		Conventional	Unspecified	345	(5–975)	EFSA (2008)		
		Conventional	Unspecified	841		Temme et al. (2011)		
		Conventional	Unspecified	91		Maynard et al. (1976)		
		Conventional	Unspecified	201	(55–376)	Tamme et al. (2006)		
		Conventional	Unspecified	2943	(28–6550)	EFSA (2008)		
Bulb vegetables								
Garlic	<i>Allium sativum</i> L.	Conventional	Aug	34	(ND–137)	Santamaria et al. (1999)		
		Conventional	Summer	180		Zhou et al. (2000)		
		Conventional	Nov–Mar	116	(3–211)	Chung et al. (2003)		
		Conventional	Apr–Oct	129	(1–462)	Chung et al. (2003)		
		Conventional	Unspecified	69	(8–161)	EFSA (2008)		
		Conventional	Unspecified	14		Maynard et al. (1976)		
Onion	<i>Allium cepa</i> L.	Conventional	Unspecified	28	(0–508)	Greenwood et al. (1986)		
		Conventional	Unspecified	127	(20–240)	Fytianos and Zarogiannis (1999)		
		Conventional	Jan–Aug	32	(5–115)	Santamaria et al. (1999)		
		Conventional	Winter	29	(28–30)	Ysart et al. (1999)		
		Conventional	Fall	134	(77–192)	Ysart et al. (1999)		
		Conventional	Summer	17	(13–21)	Ysart et al. (1999)		
		Conventional	Summer	28		Zhou et al. (2000)		
		Conventional	Summer	279	(42)	Zhou et al. (2000)		
		Conventional	Nov–Mar	14	(4–49)	Chung et al. (2003)		
		Conventional	Apr–Oct	29	(1–123)	Chung et al. (2003)		
		Conventional	Unspecified	55	(30–92)	Tamme et al. (2006)		
		Conventional	Unspecified	164	(1–638)	EFSA (2008)		
Tassel hyacinth	<i>Leopoldia comosa</i> L.	Conventional	Unspecified	59		Temme et al. (2011)		
		Conventional	Jan, Apr	53	(ND–100)	Santamaria et al. (1999)		
Roots, Rhizomes, and Tubers								
Artichoke	<i>Cynara cardunculus</i> var. <i>scolymus</i> (L.) Fiori	Conventional		174	(1–375)	EFSA (2008)		
Beetroot	<i>Beta vulgaris</i> L. var. <i>vulgaris</i> L.	Conventional	Dec–Jan	3010	± 688 (SD)	Siciliano et al. (1975)		
		Conventional	Unspecified	600		Maynard et al. (1976)		
		Conventional	Aug–Jun	480	(290–650)	Lyons et al. (1994)		
		Hydroponic	Dec	785		Lyons et al. (1994)		
		Organic	Dec	507		Lyons et al. (1994)		
		Conventional	Unspecified	1211	(224–1877)	Ysart et al. (1999)		
		Organic	Unspecified	452		Yordanov et al. (2001)		
		Conventional	Unspecified	1446	(214–3556)	Tamme et al. (2006)		
		Conventional	Unspecified	235		Herencia et al. (2007)		
		Organic	Unspecified	249		Herencia et al. (2007)		
		Conventional	Unspecified	1379	(110–3670)	EFSA (2008)		
		Conventional	Unspecified	441	(112–1820)	Greenwood et al. (1986)		
		Black salsify	<i>Scorzonera hispanica</i> L.	Conventional	Unspecified	43	(1–230)	EFSA (2008)
		Carrot	<i>Dacus carota</i> L. var. <i>sativus</i> Hoffm.	Conventional	Dec–Jan	72	± 56 (SD)	Siciliano et al. (1975)
				Conventional	Unspecified	32		Maynard et al. (1976)
				Conventional	Unspecified	68	(0–644)	Greenwood et al. (1986)
				Conventional	Aug–Jun	15	(ND–60)	Lyons et al. (1994)
				Conventional	Feb–Jun	195	(28–394)	Santamaria et al. (1999)
Conventional	Winter			115	(46–189)	Ysart et al. (1999)		
Conventional	Fall			224	(14–566)	Ysart et al. (1999)		
Conventional	Summer			34	(13–48)	Ysart et al. (1999)		
Conventional	Summer			1454	(921–1956)	Zhou et al. (2000)		
Conventional	Summer			17		Zhou et al. (2000)		
Organic	Unspecified			123		Yordanov et al. (2001)		
Conventional	Nov–Mar			373	(6–971)	Chung et al. (2003)		
Conventional	Apr–Oct	282	(1–1158)	Chung et al. (2003)				
Conventional	May–Oct	264	(7–1042)	Susin et al. (2006)				
Conventional	Unspecified	148	(< 30–525)	Tamme et al. (2006)				
Conventional	Unspecified	296	(21–1574)	EFSA (2008)				

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Table 2 (continued)

Common name	Scientific name	Production system	Season	Nitrate (mg/kg FW)		Reference		
				Mean	Min-max			
Celeriac	<i>Apium graveolens</i> L. var. <i>rapaceum</i> (Mill.) Gaudin.	Conventional	Unspecified	390	(20–975)	EFSA (2008)		
Ginger	<i>Zingiber officinale</i> Roscoe	Conventional	Unspecified	901	(peeled)	Temme et al. (2011)		
		Conventional	Summer	2659	(2234–3083)	Zhou et al. (2000)		
Lotus root	<i>Nelumbo nucifera</i> Gaertn.	Conventional	Summer	70		Zhou et al. (2000)		
Parsnip	<i>Pastinaca sativa</i> L.	Conventional	Unspecified	120		Zhou et al. (2000)		
Potato	<i>Solanum tuberosum</i> L.	Conventional	Unspecified	83	(2–349)	EFSA (2008)		
		Conventional	Unspecified	42		Maynard et al. (1976)		
		Conventional	Aug–Jun	40	(ND–105)	Lyons et al. (1994)		
		Conventional	Unspecified	81	(ND–179)	Santamaria et al. (1999)		
		Conventional	Winter	203	(47–418)	Ysart et al. (1999)		
		Conventional	Summer	104	(3–331)	Ysart et al. (1999)		
		Conventional	Unspecified	346		Zhou et al. (2000)		
		Conventional	Unspecified	16		Zhou et al. (2000)		
		Organic	Unspecified	40		Yordanov et al. (2001)		
		Conventional	Unspecified	164	(32–714)	Zhong et al. (2002)		
		Conventional	Jan–May	66	(33–117)	Kyriacou et al. (2009)		
		Conventional	May–Oct	158	(2–704)	Susin et al. (2006)		
		Conventional	Unspecified	168	(10–340)	EFSA (2008)		
		Conventional	Unspecified	186		Maynard et al. (1976)		
		Conventional	Unspecified	102	(peeled–33.8%)	Temme et al. (2011)		
		Radish	<i>Raphanus sativus</i> L.	Conventional	Unspecified	402		Maynard et al. (1976)
				Conventional	Aug–Jun	392	(145–790)	Lyons et al. (1994)
Conventional	Feb, Jun			2067	(1117–2993)	Santamaria et al. (1999)		
Conventional	Summer			1626	(1170–2153)	Zhou et al. (2000)		
Conventional	Summer			2462	(1235–3599)	Zhou et al. (2000)		
Conventional	Summer			1370		Zhou et al. (2000)		
Conventional	Nov–Mar			1494	(789–2643)	Chung et al. (2003)		
Conventional	Apr–Oct			2108	(766–4570)	Chung et al. (2003)		
Conventional	Unspecified			1309	(670–1500)	Tamme et al. (2006)		
Conventional	Unspecified			967	(115–2515)	EFSA (2008)		
Conventional	Unspecified			2136		Temme et al. (2011)		
Red radish				Conventional	Unspecified	2400, 3000		Siciliano et al. (1975)
		Conventional	Summer	2177	(1041–2721)	Zhou et al. (2000)		
Black radish		Conventional	Unspecified	1271	(233–2302)	EFSA (2008)		
White radish		Conventional	Unspecified	1416	(135–3488)	EFSA (2008)		
Rutabaga (Swedish turnip)	<i>Brassica napus</i> L.	Conventional	Unspecified	118	(39–239)	Ysart et al. (1999)		
Sweet potato	<i>Ipomea batatas</i> L.	Conventional	Summer	480		Zhou et al. (2000)		
		Conventional	Unspecified	0		Maynard et al. (1976)		
		Conventional	Aug–Jun	10	(ND–90)	Lyons et al. (1994)		
Turnip	<i>Brassica rapa</i> L.	Conventional	Apr, Jun	54	(ND–161)	Santamaria et al. (1999)		
		Conventional	Unspecified	95	(10–655)	Greenwood et al. (1986)		
		Conventional	Summer	2177	(2177)	Zhou et al. (2000)		
		Conventional	Unspecified	307	(64–1062)	Tamme et al. (2006)		
		Conventional	Unspecified	663	(10–3400)	EFSA (2008)		
		Conventional	Unspecified	1018		Temme et al. (2011)		
Fungi								
Mushroom	<i>Agaricus bisporus</i> (J.E. Lange) Imbach	Conventional	Unspecified	63		Siciliano et al. (1975)		
		Conventional	Summer	38	(1–65)	Zhou et al. (2000)		
		Conventional	Unspecified	61	(31–100)	EFSA (2008)		
		Conventional	Unspecified	58		Temme et al. (2011)		
Fruits								
Apple	<i>Malus pumila</i> Mill.	Conventional	May–Oct	3.3	(0.2–15)	Susin et al. (2006)		
		Conventional	Unspecified	11		Temme et al. (2011)		
Banana	<i>Musa spp.</i>	Conventional	Unspecified	45		Hord et al. (2009)		
		Conventional	Unspecified	153	(peeled–61.9%)	Temme et al. (2011)		
Grape	<i>Vitis vinifera</i> L.	Conventional	May–Oct	5.6	(0.5–19)	Susin et al. (2006)		
		Conventional	Unspecified	46		Temme et al. (2011)		
Kiwi	<i>Actinidia spp.</i>	Conventional	Unspecified	0	(peeled)	Temme et al. (2011)		
Nectarine	<i>Prunus persica</i> (L.) Batsch var. <i>nucipersica</i> (Borkh.) C.K.Schneid	Conventional	Unspecified	12		Temme et al. (2011)		
Peach	<i>Prunus persica</i> (L.) Batsch	Conventional	May–Oct	4	(ND–12)	Susin et al. (2006)		
		Conventional	Unspecified	10		Temme et al. (2011)		
Pear	<i>Pyrus</i> L.	Conventional	May–Oct	2.8	(1.4–4.5)	Susin et al. (2006)		
		Conventional	Unspecified	14		Temme et al. (2011)		
Orange	<i>Citrus sinensis</i> L.	Conventional	Unspecified	8		Hord et al. (2009)		
		Conventional	Unspecified	13		Temme et al. (2011)		

ND = not detected; SD = standard deviation.

Table 3
Classification of vegetables, fruits, herbs and mushrooms according to the nitrate content of edible products.

Category	Very low (< 200 mg/kg FW)	Low (200–500 mg/kg FW)	Medium (500–1000 mg/kg FW)	High (1000–2500 mg/kg FW)	Very high (2500–5000 mg/kg FW)	Extremely high (> 5000 mg/kg FW)
Herbs				Borage Coriander Parsley	Basil Chives Dill Thyme	
Brassica vegetables	Brussels sprouts Cauliflower Savoy cabbage	Broccoli Red cabbage	Broccoli raab Cabbage Curly Kale	Bok choy Chinese cabbage Kohlrabi Mustard greens Swiss chard		
Leafy vegetables		Chicory (radicchio) Fennel (leaves) Green onion (scallion)	Dandelion Endive (escarole) Lettuce, Iceberg	Amaranth Beet Belgian endive Leaf chicory Lettuce Lettuce, Butterhead Lettuce, Romaine Spinach	Sea beat Watercress	Arugula (rocket)
Stem vegetables	Asparagus	Asparagus chicory Leek	Garlic bolt Rhubarb	Celery Fennel (stem) Ginger		
Roots, rhizomes, and tubers	Artichoke	Beetroot, Red	Beetroot			
	Black salsify Lotus root Parsnip Potato Sweet potato	Carrot Rutabaga (Swedish turnip)	Celeriac	Radish, Red Radish, Black Radish, White Turnip		
Legumes	Green bean Pea Soybean sprout	Bean String bean	French bean Kidney bean			
Fruit vegetables	Gherkin Melon Pepper, Chili Pepper, Green Squash, Acorn Strawberry Tomato	Cucumber Eggplant (aubergine) Gourd, Towel Gourd, Wax Marrow Pumpkin Squash, Zucchini (courgette)	Squash, Butternut			
Bulb vegetables	Watermelon Garlic Onion Tassel hyacinth					
Fruits	Apple Banana Grape Kiwi Nectarine Peach Pear Orange					
Fungi	Mushroom					

nitrate concentration was found in the petioles vs. laminae, but also in the proximal vs. distal part of the laminae (Konstantopoulo et al., 2010). Nitrate content in the petioles of plants forming storage organs (roots, rhizomes, and tubers) tends to decrease as the crop approaches harvest maturity (Renseigné et al., 2007). This decline of nitrate concentrations in the petiole is associated with the translocation of assimilated nitrogen to the developing storage organ, along with the gradual decrease in the available soil nitrogen (Maynard et al., 1976). However, hypogeal storage organs, such as roots, rhizomes, and tubers, accumulate relatively small nitrate concentrations. Likewise, plants that develop fruits and seeds tend to accumulate less nitrate in those organs because reproductive organs are supplied with amino acids through the phloem (Blom-Zandstra, 1989; EFSA, 2008). Santamaria et al. (1999) listed plant organs in decreasing order of nitrate content as follows: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed. Through our extensive literature review, we have identified nearly 100 vegetable and fruit crops with reported nitrate content in edible plant parts; these comprise 26 leafy vegetables, 7 herbs, 7 stem

vegetables, 14 roots and tubers, 8 seed vegetables, 17 fruit vegetables, 4 inflorescence and bud vegetables, 3 bulb vegetables, 8 fruits and 1 fungus (Table 2). We have further refined the classification of vegetables, fruits, herbs and mushrooms according to the average nitrate content of their edible product (Table 3). Horticultural crops can be listed accordingly in descending order of nitrate content as follows: > herbs > leafy vegetables > stem vegetables > roots and tubers > fruit vegetables > seed vegetables (not including Kidney bean and French bean) > inflorescences and buds > bulbs > fruits > fungi.

Nitrate accumulation is also influenced by the age of plant tissue. It is well documented that younger leaves accumulate less nitrate than older leaves (Maynard et al., 1976). Higher concentration of nitrates is normally observed in the mature outer leaves of lettuce heads, where concentration could measure up to five times higher than that of the inner leaves (Abu-Rayyan et al., 2004; Konstantopoulo et al., 2010; Santamaria et al., 1999). Also, young rocket leaves accumulate significantly lower nitrates compared to fully expanded and to mature older leaves (Koukounaras et al., 2007). Similarly, nitrate concentration

in spinach harvested at later stages of plant growth was higher than that harvested at earlier stages (Maynard et al., 1976; Santamaria et al., 1999, 2001). In fact, nitrate concentrations in many leafy vegetables can be remarkably reduced when harvested as baby greens (Anjana and Iqba, 2007).

From a botanical point of view, the families *Amaranthaceae* (*Chenopodiaceae*), *Apiaceae* (*Umbelliferae*), *Asteraceae* and *Brassicaceae* are commonly known for accommodating nitrate-accumulating vegetable species (Santamaria, 2006), many of which represent important vegetable crops: amaranthus, spinach, Swiss chard in *Amaranthaceae*; celery, fennel, and herbs such as coriander, dill and parsley in *Apiaceae*; and rocket, mustard, radish, watercress in *Brassicaceae*. The mint family *Lamiaceae*, which includes many widely cultivated culinary herbs, such as basil, mint, rosemary, oregano and thyme, has not been recognized as a high nitrate-accumulating family; however, basil may potentially accumulate nitrate levels exceeding 5000 mg/kg FW (EFSA, 2008). Likewise, the European Food Safety Agency (EFSA, 2008) emphasized that certain leafy herbs like mint, oregano and thyme may contain nitrate levels over 5000 mg/kg FW, although individualized data on crops were not presented. While a number of studies address nitrate accumulation in vegetables, only a limited number addresses the issue of nitrate accumulation in fruit tree crops. It is apparent that fruits accumulate the lowest nitrate levels within the range of examined agricultural products, with the average nitrate contents being lower than 15 mg kg⁻¹ FW, except from banana, an herbaceous plant, which may accumulate up to 100 mg kg⁻¹ FW (Dejonckheere et al., 1994; Mengel and Kirkby, 1987; Susin et al., 2006). Direct contribution of vegetables, fruits and herbs to nitrite intake is relatively low, as nitrite is highly cytotoxic to plant tissues and therefore regulated *in vivo* to minimal and often non-detectable levels (Riems and Heldt, 1992). Overall, the pre-harvest occurrence of nitrites in horticultural crops is infrequent and erratic with no consistent genetic or physiological pattern yet identified.

3. Genotypic variation in nitrate accumulation

Variation among intraspecific cultivars has been reported for nitrate accumulation in leaves. Several studies have demonstrated that morphological differences among cultivars are related to nitrate accumulation in lettuce (EFSA, 2008; Santamaria et al., 1999), with crisphead lettuce cultivars accumulating higher concentrations of nitrates than iceberg and romaine lettuce cultivars. In a recent study on 48 different lettuce accessions, Burns et al. (2011) reported that average nitrate concentrations varied between morphotypes and were higher in butterhead and leaf lettuce and lower in crisphead and cos cultivars in both winter and summer growing seasons. Cantliffe (1972a, 1972b) observed significant differences in the nitrate content between smooth and savoy-leaved varieties of spinach. Mott and Steward (1972) explained that this variation was derived from nitrate's role as an osmoticum for maintaining turgor and driving leaf expansion under circumstances where photosynthetic capacity is too low to produce organic solutes. Blom-Zandstra and Eenink (1986) supported their hypothesis in studies with lettuce where they documented variation in nitrate accumulation among seven lettuce genotypes of different origin. High nitrate accumulating cultivars converted less nitrogen to proteins than the low nitrate accumulating ones, while producing less dry matter content. Reinink et al. (1987) found a strong negative correlation between shoot nitrate content and shoot dry matter content in butterhead lettuce cultivars, and a weak positive correlation between shoot nitrate content and fresh weight was found although the results were not consistent across lettuce types. Cardenas-Navarro et al (1999) viewed that nitrate concentration in fresh plant tissues, *i.e.* the ratio between nitrate and water contents, is regulated in conformity to plant homeostasis for nitrates, which is affected by genetic and environmental factors. They found a linear relationship between nitrate and water contents in tomato genotypes, which was consistently observed in plant parts as well.

Similarly, Qiu et al (2014) found a positive linear relationship between nitrate accumulation and water content in nearly 30 spinach cultivars and 30 rape cultivars. Burns et al. (2011) also reported a positive relationship between nitrate and water content for each morphotype of lettuce. In a recent study, Razgallah et al (2017) used 4 lettuce cultivars (Vitalia - type Butterhead; Great Lakes - type Iceberg; L01 - type Iceberg; L02 - type Romaine) to determine expression patterns of the nitrate transporter gene *LsNRT* in relation to nitrate accumulation; they found that high nitrate accumulator cultivars possess higher nitrate transporter transcripts than low accumulators, indicating stronger ability to take up and transport nitrates in the higher than the low accumulators. Variability in nitrate accumulation can derive not only from differences in uptake but also in the efficiency of its chemical reduction (nitrate assimilation). Luo et al., (2006) compared two contrasting pakchoi cultivars in regard to nitrate accumulation pattern. They found that Shanghaiqing, a low nitrate accumulating cultivar, had a significantly higher nitrate reductase activity and gene expression of the putative nitrate reductase compared to Liangbaiye 1, high nitrate accumulating cultivar. However, Burns et al. (2011) reported that genotypic effects on nitrate accumulation in lettuce were caused more by differences in nitrate uptake than by differences in their capacity to chemically reduce nitrate. Overall, the natural genetic variation in nitrate accumulation capacity and the linked variation in key associated traits suggest that breeding for low nitrate content in leafy vegetables can be a viable approach to reducing nitrate levels at harvest. Burns et al., (2011) reported considerable consistency in the nitrate accumulation ranking of lettuce accessions within the same morphotype between the winter and summer seasons; these findings indicated that screening for nitrate accumulation among lettuce cultivars or breeding lines of the same morphotype or seasonal type can be carried out effectively regardless of growing season.

4. Cultural practices affecting nitrate accumulation

4.1. Nitrogen fertilization

Nitrogen fertilization, being the primary nitrate source for plant uptake, is the major driver of nitrates in edible crops. In general, increasing nitrogen fertilizers resulted in higher yields but the nitrate content in the edible parts of the crops also increased when higher rates of nitrogenous fertilizers were applied. Application of high nitrogen rate not only increases the risk of nitrate accumulation in crops, moreover it increases production cost and raises the risk of water quality deterioration due to nitrate leaching. For example, corn yield increased by 4% in response to 30% increase in nitrogen application rate, but 53% of the nitrate was lost through leaching. The yield decreased by 10%, when the nitrogen application rate was reduced to 30%, while nitrogen loss through leaching was reduced by 37% (Donner and Kucharik, 2003). Because nitrogen requirements of crops decrease as plant growth matures, it is not surprising that excessive applications of nitrate based-fertilizers at late stages of vegetative growth have a strong impact on nitrate accumulation in leafy vegetables. For this reasons, an effective strategy for reducing nitrate concentration in plants is to deprive them of nitrogen for few weeks before harvesting, during which process nitrates are removed from the vacuoles and the plant stores organic compounds to replace the declining osmoticum (Blom-Zandstra and Lampe, 1983). The use of nitrate-free solution few days before harvest has been successfully applied in leafy vegetables grown under soilless culture (Borgognone et al., 2016). The accumulation of nitrates in the edible parts of crops is also directly related to the type of nitrogen fertilizer used (Gunes et al., 1995;). Nitrogen fertilizers can contain nitrogen as nitrate, ammonium, urea or other organic forms. Although nitrogen forms had no significant effect on the yields of Peking cabbage and spinach, they remarkably influenced nitrate concentrations: nitrate-N increased nitrate concentration much more than did ammonium-N (Wang and Li, 2004). The use of

fertilizers based on ammonia or a mixture of nitrate and ammonium can reduce the nitrate content in plants (Inal and Tarakcioglu, 2001; Santamaria et al., 2001). Santamaria and Elia (1997) reported that the replacement of nitrate with ammonium in the recirculating solution reduced the nitrate accumulation in hydroponically-grown endive. Depending on which plant part is to be consumed, appropriate fertilizer can be also selected to reduce nitrate accumulation (Zhou et al., 2000), e.g. the order of nitrate accumulation in kale leaves in response to the application of different nitrogenous fertilizers was: urea > ammonium carbonate > ammonium nitrate > ammonium sulfate; for nitrate accumulation in petioles, it was: urea > ammonium nitrate > ammonium sulfate > ammonium carbonate (Renseigné et al., 2007). Once nitrogen fertilizers are applied to the soil, fertilizers are absorbed directly by plants or converted into various other forms through the oxidation process before plant uptake. Moreover, the release of nitrogen in organic fertilizers is slower than that in inorganic fertilizers since organic fertilization typically does not provide nitrogen in a readily accessible form (Herencia et al., 2011). For this reason, nitrate accumulation in edible part of crops is usually lower in organically grown crops than in conventionally grown crops (Barker, 1975; Lairon et al., 1984; Muramoto, 1999; Pussemier et al., 2006; Pavlou et al., 2007; Schuphan, 1974; Temperli et al., 1982; Vogtmann et al., 1984), although this finding was not consistent with other studies (De Martin and Restani et al., 2003; Guadagnin et al., 2005). Dangour et al. (2009) surveyed a total of 52,471 articles published during 50 years from 1958 to 2008, and identified 162 studies (137 crops and 25 livestock products) and only 55 studies among those showed positive effects of organic production system on crop quality. They also found that conventionally produced crops had a significantly higher content of nitrogen, compared to organically produced crops. In a survey of nitrate and nitrite concentrations in raw vegetables (broccoli, cabbage, celery, lettuce, and spinach) collected from 5 major cities in the United States, Nuñez de González et al. (2015) found that conventional vegetables contained significantly higher nitrates compared to organic vegetables but there were no differences in nitrite between conventional and organic vegetables, where nitrite ranged from 0.1 to 1.2 mg/kg FW with the exception of conventional spinach that contained 8.0 mg/kg FW. Organically grown baby greens, such as green lettuce red lettuce, watercress, rocket, chard, and corn salad contained significantly less nitrates at harvest compared to conventionally grown ones (Aires et al., 2013). Woese et al. (1997) reviewed 41 comparative studies addressing nitrate content in vegetables from conventional and organic production systems and concluded that there were clear findings confirming a lower nitrate content in organically grown vegetables than conventionally cultivated crops mainly of leaf, root and tuber vegetables. Meanwhile, in other vegetables with a lower nitrate accumulating potential, such as fruit, seed and bulb vegetables, the results demonstrated either no differences in nitrate content based on production system, or organically grown ones had lower nitrate levels. Nitrate concentrations in apples and strawberries were very low on the whole and in some cases they were below the detection limit. No significant differences were found between conventional and organic fruits (Reinken et al., 1990). A comparative review by Worthington (2001) based on the results of 18 studies, demonstrated that out of 176 comparisons made for nitrate levels of organic and conventional fruits, vegetables and grains, 127 cases showed lower nitrate levels in organic crops, while 34 showed higher nitrate levels and 6 did not show any differences compared to conventional crops. The higher nitrate accumulation found in some organically produced crops can result from an excessive N-NO₃ released too late in the growing season (Nazaryuk et al., 2002).

4.2. Fertilization with other macronutrients and trace elements

Besides nitrogen, other nutrients can also influence the nitrate accumulation in crops. Phosphorus deficiency affects negatively nitrate uptake in sugar beets (Hills et al., 1970), and nitrate concentration in

lettuce leaves (Buwalda and Warmenhoven, 1999). Sulfur deficiency was associated with nitrate accumulation in mung bean leaves (Arafa and Luthra, 1972) as nitrate reductase activity entails a sulfhydryl group. Sulfur deficiency also leads to the nitrate accumulation in lettuce (Blom-Zandstra and Lampe, 1983). Field studies and work on nutrient solutions in hydroponic crops have shown little effect of moderate K deficiency on nitrate accumulation in vegetables, although increased application of K stimulates nitrate uptake and transport of nitrate to the aboveground parts of plants, and further promotes nitrate assimilation (Ahmed et al., 2000). The effect of Ca supply on nitrate accumulation in vegetables is still not clear. Calcium has an effect on nitrate uptake and possibly an effect on its reduction by plants. Calcium deficiency restricts root development and thus may indirectly affect nitrate uptake efficiency. No evidence was found that Mg influences nitrate accumulation, apart from its effects on general plant metabolism and chloroplast development (Kessler, 1964) and even though nitrite reductase activity is mainly dependent on the function of PS I (Riens and Heldt, 1992). Chloride in the soil is antagonistic to nitrate uptake as both ions play an interchangeable role in osmoregulation (Veen and Keilenendorst, 1986). Chloride inhibits excessive nitrate uptake by substituting its osmoticum role. Several studies reported that high concentrations of chloride in the root zone decreased the net nitrate uptake and nitrate translocation to the shoot in many vegetable species such as artichoke and lettuce grown hydroponically (Borgognone et al., 2016; Neocleous et al., 2014; Urrestarazu et al., 1998). Rubinigg et al. (2003) demonstrated that in the presence of high chloride levels, root-to-shoot translocation of nitrate could be reduced at the site of entrance into xylem parenchyma cells through competition for the same channel. Therefore, chloride application may constitute a strategy for reducing nitrate contents in high nitrate accumulating vegetables, (Borgognone et al., 2016) in hydroponic crops. On the other hand, the efficiency of this strategy is restricted by the toxicity of chloride at high concentrations as well as by the sensitivity of the species to chloride (Rouphael et al., 2018a, 2018b).

Molybdenum is a component of nitrate reductase and thus a prerequisite for the enzymatic reduction of nitrate to nitrite. In Mo-deficient plants, nitrate may accumulate to very high levels in the leaves, sometimes exceeding 3% on a dry weight basis (Hewitt and Smith, 1975). Plants relying solely on ammonium as nitrogen source apparently do not have a Mo requirement (Hewitt and Gundry, 1970). Iron has a role in nitrate reductase in that reduced ferredoxin formed by illuminated chloroplasts participates in the reduction. For this reason, Fe deficiency decreased nitrate reductase activity of cucumber at the root and leaf level (Borlotti et al., 2012).

4.3. Plant biostimulants

Nitrate accumulation can also be affected by the application of so called 'plant biostimulants' which include natural substances (e.g., humic acids, protein hydrolysates, seaweed extracts) and microbial inoculants (e.g., arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria) (Colla and Rouphael, 2015; Colla et al., 2014, 2017a, 2017b; De Pascale et al., 2017; Rouphael et al., 2015, 2017b, 2017c, 2017d). Several reports showed that application of biostimulants can reduce nitrate accumulation depending on the crop, environmental conditions and biostimulant applied. For instance, application of protein hydrolysates decreased nitrates in leafy vegetables such as lettuce, rocket, radish, Swiss chard, and spinach (Colla et al., 2013a, 2013b, 2015). Tsouvaltzis et al., (2014) reported a nitrate reduction of 29% in greenhouse lettuce after protein hydrolysate application. The above findings were linked to biostimulant effects in down-regulating the expression of genes encoding for transporters involved in root nitrate uptake or to an up-regulation of the main N assimilation genes (e.g. nitrate reductase genes) leading to a more efficient assimilation of nitrates into amino acids. Similarly, foliar treatments with seaweed extract increased the nitrate reductase activity and thus the protein

content in leaves of eggplant (Ramya et al., 2015). Moreover, inoculation of lettuce plants with plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi (*Glomus intraradices*), alone or in combination, stimulated significantly nitrate reductase activity (Kohler et al., 2008) diminishing nitrate accumulation in the plant. On the contrary, an increase of nitrate accumulation was observed in lettuce plants inoculated with *G. intraradices* and fertilized with urea (Mitova et al., 2017). This highlights the potential interaction between biostimulant application and nitrogen fertilization that must be considered in defining strategies for reducing nitrate accumulation in the edible parts of crops.

5. Climatic factors affecting nitrate accumulation

5.1. Light conditions

A close correlation between light intensity and nitrate content in vegetables has been implicated in a number of studies. The nitrate uptake and assimilation depends on irradiance and photosynthesis since this provides the carbon skeletons necessary to integrate the ammonium derived from nitrate reduction and the electrons required for the reduction of nitrate to nitrite, which is initiated by nitrate reductase (Cavaiuolo and Ferrante, 2014). Therefore, nitrate accumulation in the plant tissues varies with the season (Santamaria, 2006) and is often stimulated in autumn-winter period where low light conditions prevail than in spring growing season (Santamaria et al., 1999). Reduced nitrate reductase activity can be one of the causes for this as its activity is highly regulated under intense light conditions (Pilgrim et al., 1993). For instance, Gaudreau et al. (1995); Chadjaa et al. (1999) as well as Fallovo et al. (2009) showed that a reduction in irradiance intensity was accompanied with reduced nitrate reductase activity and increased nitrate accumulation in several important leafy vegetables such as lamb's lettuce, spinach and *Lactuca sativa* L. var. *acephala* cv. Green Salad Bowl. Similarly, Proietti et al. (2004) showed that the five weeks-spinach grown under 10 h light/14 h dark photoperiod and with a photon flux of $200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ had a 65% higher nitrate concentration compared to plants grown at $800 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. A putative mechanism behind the accumulation of nitrate could be associated to the fact that nitrate is a favored osmoticum for maintaining turgor pressure in leafy vegetables under low irradiance conditions, replacing energy-expensive carbohydrates (Blom-Zandstra and Lampe, 1985; Buwalda and Warmenhoven, 1999). However, Cantliffe (1972a), observed a minimal effect of light above irradiance levels of $450\text{--}900 \mu\text{mol m}^{-2} \text{s}^{-1}$. Similarly, Cantliffe (1972b) and Parks et al. (2008) were able to demonstrate that the leaf nitrate concentration in Swiss chard was mainly affected by N supply and to a lesser extent by light level, because the light conditions exceeded the critical level ($160\text{--}200 \mu\text{mol m}^{-2} \text{s}^{-1}$). In a recent study Fu et al. (2017) investigated the effects of three combinations of light intensity (60, 140 and $220 \mu\text{mol m}^{-2} \text{s}^{-1}$) and three N concentrations (7, 15 and 23 mmol L^{-1}) on nitrate accumulation in lettuce cv. youmaicai. The authors reported that the maximal nitrate concentration was recorded at the low light intensity and high N of 23 mmol L^{-1} indicating that high irradiance provides more carbohydrate and photochemical energy to stimulate nitrate assimilation into amino acid (Konstantopoulou et al., 2010). Based on the previous studies, the effect of light intensity on nitrate accumulation seems species-dependent; therefore, field as well as greenhouse studies addressing Genotype \times Environment \times Management (G \times E \times M) interaction are required to explore the ideal combination on genotype/species, environment (light intensity) and management strategy (nitrogen fertilization) in order to harvest vegetables with low nitrate concentrations.

In addition to light intensity, the time of harvest during the day appears to have a transient, but significant effect on nitrate levels in a wide range of leafy vegetables (Chang et al., 2013a, 2013b; Colonna et al., 2016). For example Chang and co-workers carried out a series of

experiments under open field and growth chamber conditions on the fluctuation of nitrate and nitrite concentrations over a 24 h light/dark cycle for spinach, sweet basil and scallions (Chang et al., 2013a, 2013b). They reported that nitrate and nitrite concentrations fluctuated over the 24 h period, and these changes were significantly correlated to the light intensity at harvest and were also species-dependent. The highest nitrate levels in spinach were recorded at 9:30 a.m., which was around 3 h prior to an increase in light intensity, whereas the lowest values were observed around 3–6 h later (Chang et al., 2013a). The nitrate and nitrite concentrations for scallions were all relatively low with the highest nitrate values observed prior to sunrise, indicating a species-dependent phenomenon. Therefore, additional in-depth studies should be carried out to assess the species-specific response to the 24 h light/dark cycle of different leafy vegetables. Moreover, Colonna et al. (2016) showed that the nitrate content in ten greenhouse leafy vegetables (chicory, green lettuce, lamb's lettuce, mizuna, red chard, red lettuce, rocket, spinach, Swiss chard and tatsoi) was higher by 36.5% in plants harvested under morning low light conditions ($200\text{--}400 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 8:30 a.m.) compared to those harvested under midday conditions of high photosynthetically active radiation ($800\text{--}1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 2:30 p.m.). A similar trend in nitrate accumulation was also observed in leafy mustards harvested at low irradiance intensity (Makus and Lester, 2004). Taken all together, growers can reduce the nitrate content of raw vegetables by avoiding harvesting during dull weather conditions and by harvesting during the time of the day that the lowest nitrate content is prognosticated.

Light quality demonstrates more complex effects than light intensity in regulating nitrate accumulation in leafy vegetables (Bian et al., 2015). For instance, Ohashi-Kaneko et al. (2007) reported that the nitrate concentration was significantly reduced in hydroponically-grown lettuce under irradiation from blue fluorescent lamps alone or in combination with blue and red fluorescent lamps. In the same paper, the authors showed that in the cultivation of spinach, irradiation from red fluorescent lamps can produce plants with decreased nitrate content, whereas no significant effect of the four light quality treatments (blue, red, mixture blue and red and white light) was recorded on nitrate content of komatsuna plants, indicating that the influence of light quality on this undesirable component is species-dependent (Ohashi-Kaneko et al., 2007). Furthermore, Lin et al. (2013) found that a mixture of blue, red plus white LED was more effective in reducing nitrate concentration by 20.5% and 32.5% in soilless Boston lettuce (*Lactuca sativa* L. var. *capitata*) than fluorescent lamp (control treatment) and a mixture of red and blue LED, respectively. Similar results were also obtained by Liu et al. (2016) since the cool white fluorescent light (T5) as well as the wide spectrum high-pressure sodium light (HPS) reduced nitrate content in lettuce by 62% compared to the mixture of red and blue (RB) LED light. A presumed mechanism involved in the reduction of nitrate might be that broad spectral composition stimulated nitrate assimilation by contributing to protein synthesis, thus reducing the accumulation of nitrates in lettuce plants (Liu et al., 2016). In addition, several authors (Urbanavičiute et al., 2007; Wen et al., 2009; Zhou et al., 2013) have demonstrated the effectiveness of modifying the red-to-blue ratio (*i.e.* R/B ratio of 4:1 or 8:1) in reducing nitrate levels of hydroponically cultivated lettuce. Obviously, the effect of spectral quality (wavelength) on nitrate uptake and accumulation is far from being fully elucidated. Thus, future research is warranted to assess the physiological and molecular changes linked to these modifications and also for identifying the optimal light intensity and quality that can be strategically applied to reduce nitrate accumulation in leaf tissues.

5.2. Air temperature

There is limited research in the scientific literature addressing the direct and indirect effects of temperature on nitrate accumulation in horticultural crops. Nitrate uptake and accumulation is sensitive to temperature and it depends on both environment as well as root-zone

temperature (Bose and Srivastava, 2001). Temperatures negatively affecting plant growth can also encourage nitrate accumulation (Habben, 1973). Crop season affects plant growth more than nitrate accumulation: late-spring season favorably affects biomass production compared to late-autumn season wherein plants more moderately respond to N levels, leading to a large variation in plant biomass and N contents in the leaves (Pavlou et al., 2007). In seasonal experiments, Parks et al. (2008) reported higher growth and greater nitrate accumulation in the spring (18–39 °C) compared to winter (14–28 °C). Similarly, the higher nitrate accumulation (by 22%) observed in *Lactuca sativa* L. var. *acephala* during the summer season could be associated with the higher range of temperatures (19.3–29.6 °C) compared to the spring season (16.2–22.7 °C) (Falovo et al., 2009). However, high temperatures do not necessarily contribute to an increase in the nitrate concentration in horticultural crops. In fact, under high temperature (> 30 °C), Cantliffe (1972c) showed that spinach growth and nitrate accumulation in leaf tissue decreased. Finally, it must be pointed out that under greenhouse conditions (especially unheated greenhouses) there is a strong positive correlation between radiation and air temperature making it difficult in many cases to establish a direct cause-effect linkage between single microclimate factor (light or temperature) and nitrate change in plant tissues.

5.3. Carbon dioxide enrichment

The role of high carbon dioxide in limiting the concentration of nitrate in food vegetables has not been widely investigated in the scientific literature. It is well established that excessively high level of carbon dioxide (CO₂) can inhibit N assimilation, thus limits photosynthate flow from leaves (Dong et al., 2017). Proietti et al. (2013) investigated the influence of the factorial combination of two light intensities (200 or 800 μmol m⁻² s⁻¹) and two CO₂ concentrations (360 or 800 ppm) on the quality of spinach including the detrimental metabolite nitrate. Particularly interesting is that growing spinach at high CO₂ concentration caused a significant reduction of the nitrate concentration in leaves compared to growth at normal CO₂, particularly under low light conditions. The authors highlighted in their study the importance of considering the interaction between light intensity and CO₂ concentration in decreasing nitrate accumulation in leafy vegetables.

6. The postharvest fate of nitrates and nitrites

The postharvest quality and safety of fresh vegetable crops, particularly of salad crops, relates to their residual nitrate content that furnishes the formation of nitrite through salivary enzymes, which in turn is implicated in the formation of nitrosamines through reaction with free amines, and in the endogenous formation of carcinogenic N-nitroso compounds (Duncan et al., 1997; Kyriacou and Rouphael, 2018; Walker, 1990). Residual nitrates at harvest are defined at large by genotypic and agroenvironmental factors. Preharvest nitrate accumulation in vegetables is directly dependent on the uptake of nitrate ion in excess of its reduction and subsequent assimilation. Comparable levels of nitrate and nitrite reductase activities during the growth period prohibit the accumulation of considerable levels of nitrites. However, the transport of nitrates taken up by the roots through the transpiration stream to the leaves, where assimilation takes place, is interrupted after harvest and so is the diurnal variation in leaf nitrates corresponding to fluctuation in the transpiration stream (Riens and Heldt, 1992). Potential reduction of nitrates postharvest may only utilize substrates already accumulated in the cytosol and vacuole, hence an intriguing facet of the postharvest life of vegetables is the putative endogenous conversion of nitrate residues to nitrites, and how storage conditions might elicit or inhibit this conversion.

During the storage of fresh vegetables, potential changes in their nitrate content are highly dependent on temperature, being more likely

under ambient temperature than in cold storage (Alexander et al., 2008; Ekart et al., 2013). Several reports attest to the inhibition of postharvest nitrate reduction under cold storage but the effective temperature range seems a function of species and storage duration. Kim and Ishii (2007) have demonstrated the lack of large or consistent changes in nitrate content of rocket (*Eruca sativa* Mill) with or without roots during dark storage for 10 days at 4 °C or 15 °C. Absence of nitrate reduction was also reported by Ferrante et al., (2003) for rocket leaves stored for 12 days in darkness at 5 °C, though intriguingly nitrite content under the same conditions decreased after 8 days of storage. Further work on rocket leaves was conducted by Koukounaras et al., (2007, 2010) who identified no consistent storage effect on nitrate content after 14 days at 0, 5 and 10 °C in the dark, or after 14 days at 8 °C in the presence of 500 μL/L ethylene. Similarly, Chung et al., (2004) found no significant changes in the nitrate and nitrite contents of spinach, crown daisy, Chinese spinach and non-heading Chinese cabbage stored for seven days at 5 °C. Also, nitrate concentrations within the leaves of Cos lettuce (*Lactuca sativa* L. cv. Parris Island) were retained during 10-day storage at 5 and 10 °C without significant conversion to nitrite, although the nutritional value and market quality had decreased (Konstantopoulou et al., 2010). Similarly, no significant change in the nitrate contents of romaine type lettuce (cv. Paris Island) and leaf type lettuce (cv. Atraxion) were observed during 15 days of storage at 1 °C (Siomos et al., 2002), or of crisphead lettuce (cvs. Marius and Saladin) stored at 1 °C for 14 days, though other quality components, such as vitamin C and soluble carbohydrates had declined with storage (Poulsen et al., 1995). Limited increase was found in the nitrate contents of root crops (celeriac, carrot, parsnip) stored at 0 °C for up to 180 days which can be explained on the basis of parallel fresh weight loss (Ilić and Sunić, 2015).

Whereas cold storage appears prohibitive to changes in nitrate and nitrite levels, storage at ambient temperature can have a profound effect on them, associated however with the overall quality of the stored products. When short-petioled cabbage (*Brassica chinensis* L.), long-footed cabbage (*Brassica chinensis* L. var. *communis* Tsen and Lee), field mustard (*Brassica campestris* L.), broad-leaf mustard (*Brassica juncea* L. var. *rugosa* (Roxb.) Kitam.) and water convolvulus (*Ipomoea aquatica* Forsk) were kept in plastic bags for up to three days at -10, 2, 15 or 26 °C distinctive decrease in nitrate levels was observed only in cabbage and mustard samples stored at 26 °C (Lin and Yen, 1980); notably, samples at this temperature deteriorated rapidly and were considered unmarketable after 2 days and unusable after 3 days. Increase in nitrite levels of the same samples was also observed after 3 days of storage at 26 °C. Moreover, storage at -10, 2 and 15 °C did not exert significant effect on the activities of nitrate and nitrite reductases, whereas storage at 26 °C enhanced the activities of both enzymes. Similarly, Chung et al (2004) have reported the sharp postharvest decrease in the nitrate concentration of fresh spinach, Chinese spinach and non-heading Chinese cabbage (but not of crown daisy) after 3 days at 22 °C, succeeded by analogous increase in their nitrite content. However, the fresh market samples used in this study had exceedingly high initial nitrate levels (e.g., spinach mean = 5210 mg kg⁻¹ f.w.). Moreover, no information was provided on the use of packaging or the quality of the stored vegetables at the time of nitrite increase, which most likely had been unacceptable based on the authors' suggestion that nitrate reductase activity was due to microbial proliferation. Likewise, the decline in nitrates and the accumulation of nitrites in spinach leaves stored at ambient conditions, reported in an early study by Phillips (1968), was marked by inconsistency in results and lack of statistical characterization, moreover no information was given on the quality condition of the samples during storage. Indeed, high populations of facultative anaerobic bacteria were implicated in the reduction of nitrate to nitrite in exudates of whole carrots containing high concentrations of nitrite (Hicks et al., 1975). Accordingly, it might be inferred that the postharvest decrease in nitrate levels and concomitant increase in nitrites is temperature- as well as species-dependent, and

related to the initial nitrate residues, the perishability of the crop and ultimately on the quality condition of stored vegetables. Overturning the equilibrium in the levels of nitrate and nitrite reductase activities postharvest requires an increase in exogenous activity of nitrite reductase of microbial origin. Postharvest nitrite accumulation in the absence of visible quality deterioration and decay seems unlikely. Therefore, rapid cooling and optimally low temperature storage conditions are essential means for preventing postharvest nitrite accumulation as much as they are for preserving overall quality. It is not surprising that the nitrate and nitrite contents of leafy, stalk, root and tuberous vegetables remained largely unaffected during frozen storage for 12 weeks (Schuster and Lee, 1987).

Though temperature is unequivocally the most critical of storage conditions with respect to its indirect effect on postharvest nitrate reduction, another important factor that has only scarcely been investigated is light conditions during storage. Nitrate assimilation in photosynthetic cells necessitates two reductive steps, one for nitrate and another for the nitrite ions. Nitrate is reduced to nitrite by the NADPH-dependent action of nitrate reductase (NR) in the cytosol, then transported inside the chloroplast stroma where it is reduced to ammonia by nitrite reductase (Brunswick and Cresswell, 1988; Solomonson and Barber, 1990). Nitrite reductase activity is dependent on the function of PS I and is therefore light-dependent, although minor nitrite reduction may be supported by reducing agents produced by catabolic pathways (Riens and Heldt, 1992). It is therefore not surprising that the numerous studies reporting stability in the postharvest levels of nitrites were all related to dark storage. Intriguing however is the report by Ferrante et al. (2003) that nitrate content of rocket leaves declined during storage under $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ light with 12 h photoperiod, followed by moderate increase of nitrite content; however, the lack of consistency in these trends warrants further evaluation. In particular, it is difficult to explain the accumulation in nitrite under light storage given the light-dependent nature of nitrite reduction. In the absence of light, nitrite reductase activity is halted and nitrate reductase activity must be regulated to avoid nitrite accumulation at cytotoxic levels (Riens and Heldt, 1992). Nevertheless, the mechanism that coordinates the activation state of nitrate reductase present in the cytosol with photosynthetic activity in the chloroplasts is still unknown.

7. Conclusions

Notwithstanding the uncertainty surrounding claims of adverse health risks associated with dietary intake of nitrate, the EU and large-scale food retailers are enforcing low nitrate tolerances on vegetable and fruit growers. These specifications can be met through an appropriate management of cultural practices (e.g., timing, rate and form of N application), environmental conditions during plant growth (e.g., light intensity, spectral quality, photoperiod, air and root-zone temperature and carbon dioxide concentration), harvest maturity, the diurnal timing of harvest and postharvest storage conditions (temperature, light, and duration). Greenhouse production under soilless culture seems to be the most effective technique for controlling nitrate accumulation in vegetables and herbs and several protocols of nutrient solution management are currently available in this respect. Genetic variability in nitrate accumulation should also be considered in the choice of cultivar. Finally, breeding for low nitrate accumulating cultivars is an urgently needed measure, especially for crops having an inherently high nitrate accumulating capacity like leafy vegetables.

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