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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 CO_2 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 (NO_3^-) 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 NO_3^- set by the European Union (3.7 mg/kg body weight per day), equivalent to 222 mg of NO_3^- 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 (*Spinacia oleracea* L.)	–	3,500
Lettuce (*Lactuca sativa* 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 (*Eruca sativa* Mill., *Diplotaxis* sp., *Brassica tenuifolia* Baill., *Sisymbrium tenuifolium* 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 (Cavaiuolo 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 (Cavaiuolo and Ferrante, 2014; EFSA, 2008). As a precautionary measure, the European Commission regulations Nº 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; Chadjaa et al., 1999; Colonna et al., 2016; Demsar and Osvald, 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 Osvald, 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 hypogeal 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