Int. J. Curr. Res. Biosci. Plant Biol. 2016, 3(8): 74-80

EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 (Online) • Volume 3 • Number 8 (August-2016)

Journal homepage: www.ijcrbp.com

Review Article

doi: http://dx.doi.org/10.20546/ijcrbp.2016.308.012

Nitrate to Nitrite Ratio in Vegetables: A Systematic Review

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Abstract

Nitrates and nitrites occur naturally in the environment. They are important plant nutrients and vegetables account for about 70-90% of the total estimated dietary nitrate intake. Nitrate plays an important role in the nutrition and function of plants. Vegetables rich diet has been described beneficial for longevity and overall health of humans. The positive effects of vegetables may be attributed, in part, to inorganic nitrate present in green leafy vegetables. The nitrate and nitrite level in vegetables varies significantly from type of vegetables, demographic area and season. Nitrate and nitrite content of vegetables has been reviewed and an attempt was made to establish nitrate to nitrite ratio present in vegetables.

Article Info

Accepted: 09 July 2016
Available Online: 06 August 2016

Keywords

Nitrate
Nitrite
Nitrate to nitrite ratio
Plant nutrients
Vegetables

Introduction

Plants and animals require nitrogen to live and grow. However, nitrogen gas, which is abundant in the air we breathe, must first be converted to nitrogen compounds that can be used by plants and animals as sources of nitrogen. This process is called nitrogen fixation**.** Nitrate and nitrite are two of the nitrogen compounds that are used by plants and animals and eventually return to the air as nitrogen gas**.** Nitrate and nitrite can also be produced in the body. In nature, plants utilize nitrate as an essential nutrient. Nitrate and nitrite are also used in food preservation, some pharmaceutical drugs, and the production of munitions and explosives (Santamaria, 2006).

Nitrates and nitrites occur naturally in the environment. They are important plant nutrients and can be used in fertilizers. In addition, they can be added to some food products as preservatives. Vegetables account for about 70-90% of the total estimated dietary nitrate intake. Nitrate plays an important role in the nutrition and function of plants. The nitrate concentration in vegetables depends on a number of factors including species variation, season, light, temperature, method of growth, and fertilizer used. The nitrate and nitrite levels of vegetables after harvesting can be affected by the storage and processing methods (WHO, 2003).

Vegetables rich diet has been described beneficial for longevity and overall health. The positive effects of vegetables may be attributed, in part, to inorganic nitrate (NO_3^-) present in green leafy vegetables (Gilchrist et al., 2010). To elicit any biological effects NO_3^- are likely to be converted to the nitrite (NO_2^-) ion in the mouth via oral facultative anaerobic bacteria on the surface of the tongue (Duncan et al., 1995). When swallowed, NO_2^- is further converted into nitric oxide (NO). The reduction of NO_2^- to NO and other reactive nitrogen intermediates are facilitated in low oxygen state which is also called as hypoxia (Bryan, 2006). The production of NO via nitric oxide synthase (NOS) is impaired in hypoxia and, thus, it has been proposed that the NO_3^-- NO_2^--NO pathway represents a complementary system for NO generation

across a wide range of redox states (Lundberg et al., 2008). NO is an essential physiological signaling molecule with numerous beneficial functions in the body, including the regulation of blood flow, muscle contractility, glucose and calcium homeostasis, and mitochondrial respiration and biogenesis (Dejam et al., 2004).

Nitrates in vegetables have neither taste nor smell but are identified using laboratory tests. Various methods have been developed to determine nitrates in food, water and other matrices. Over the years, analytical techniques, such as spectrophotometry (Huarte-Mendicoa et al., 1997; Nagaraja and Kumar, 2002), potentiometry (Perez-Olmos et al., 1997), ion chromatography (Mcmullen et al., 2005), polarography (Ximenes et al., 2000), capillary electrophoresis (Oztekin et al., 2002) as well as high-performance liquid chromatography (Reece and Hird, 2000; Sanderson et al., 1991) have been used.

Role of nitrate and nitrite in human health

The inorganic nitrate present in certain vegetables and fruits can provide a physiologic substrate for reduction to nitrite, nitric oxide, and other metabolic products that produces vasodilation, decrease blood pressure, and support cardiovascular, metabolic and other body function. Moreover, there are enough evidence for benefits of nitrates in several areas including prevention of microbial infections, cardiovascular diseases, and reduction in the risk of gastric cancer (Santamaria et al., 2006).

It has also been established that dietary supplementation of nitrates can significantly increase the nitrite levels and reduce resting blood pressure in young adults (Bailey et al., 2010; Larsen et al., 2006). Apart from reducing the blood pressure, this dietary nitrate supplementation may have positive effects upon the physiological response to exercise (Bailey et al., 2010; Larsen et al., 2007). In a human study, supplementation with sodium nitrates (Larsen et al., 2007) or beetroot juice (Bailey et al., 2009) resulted in a significant reduction in oxygen uptake during submaximal cycling. In a recent placebo controlled study, it is reported that amaranth extract supplementation significantly increased the nitrate and nitrite levels in blood and saliva (Subramanian and Gupta, 2016). These results are important because the oxygen uptake and work rate relationship have traditionally been considered to be independent of age, health status, and aerobic fitness (Jones and Poole, 2005). The reduction in the oxygen cost of moderate intensity exercise following dietary nitrate supplementation may be a result of a decreased ATP cost of muscle force production (Bailey et al., 2010) and/or enhanced mitochondrial efficiency (Larsen et al., 2011). In a study by Stokes et al, dietary supplementation of nitrate and nitrite in mice has shown to reverse endothelial dysfunction, suppresses microvascular inflammation, and reduces level of C - reactive protein in mice subjected to a high-cholesterol diet (Stokes et al., 2009).

An Acceptable Daily Intake (ADI) for nitrate of 3.7 mg/kg b.w./day, equivalent to 259 mg nitrate per day for a 70 kg adult was established by the former Scientific Committee on Food (SCF) and was reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002 (EFSA, 2008). The JECFA has also set an ADI of 0-0.07 mg/kg b.w. for nitrite, which is equalent to 0-4.9 mg nitrite for a 70 Kg adult (FAO/WHO, 2003a,b).

Nitrate content of vegetables

Generally, Leaves contain higher levels of Nitrate whereas seeds and tubers contain lesser level. Humans are exposed to nitrate mainly through the consumption of vegetables, and to a lesser extent water and other foods. Nitrate is also formed endogenously. In contrast exposure to its metabolite nitrite is mainly from endogenous nitrate conversion (EFSA, 2008).

Nitrate is mainly to be found in cell vacuoles and is transported in the xylem. The xylem carries water and nutrients from the roots to the leaves, while the phloem carries the products of photosynthesis from the leaves to the growth points of the plant (i.e. storage organs such as seeds or tubers). This means leaf crops such as cabbage, lettuce and spinach have fairly large nitrate concentrations whereas storage organs such as potato, carrot, and pea and beans have relatively small concentrations (EFSA, 2008).

Vegetables are classified according to the nitrate content as very low (<200 mg/kg), low (200-500 mg/kg), middle (500-1000 mg/kg), high (1000-2500 mg/kg) and very high (>2500 mg/kg) (Santamaria, 2006). This classification is summarized in Table 1.

The distribution of nitrate is not even across the product, e.g. removal of stem and midrib resulted in a decrease of nitrate content by 30-40% in lettuce and spinach. On the other hand, nitrate content was found to decrease by 20

to 62% after peeling of potatoes, bananas, melons and beetroot. Nitrate is soluble in water and washing of leafy vegetables can reduce nitrate levels by 10-15% (Chung et al., 2003; EFSA, 2008).

Different studies have shown reduction of nitrate levels (16 to 79% loss) when vegetables such as peas, cabbage, beans, carrots, potatoes, spinach, endives, and celery leaves are cooked in water. For potatoes, a study found that the greatest decrease in reducing nitrate (36-58%) and nitrite (82-98%) was observed when peeled potatoes were boiled in water compared to steaming (Stopes et al., 1988). Spinach is regularly regarded as one of the highest nitrate accumulators. Its distinctive shoot structure, consisting of petioles, where nitrate passes through and accumulates, and leaf-blades, where nitrate reduction and assimilation take place, might be one of the reasons for spinach's relatively high nitrate concentration. Certain vegetables, because of a very efficient uptake system, an inefficient reductive system, or an unfavorable combination of both, tend to accumulate more nitrate than others (Maynard et al., 1976).

Table 1. Classification of vegetables on the basis of nitrate content.

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

Nitrite content of vegetables

The nitrite concentrations in fresh, undamaged vegetables are usually very low but under adverse post-harvest storage conditions nitrite concentration can increase in vegetables as a result of bacterial or endogenous nitrate reductase reducing the nitrate to nitrite. Under refrigerated storage, the endogenous nitrate reductase in vegetable is inactivated. However, high levels of nitrite have been found in homemade vegetable purees even after refrigerated storage for only 12 hours or more. Presumably pureeing releases endogenous nitrate reductase causing excessive formation of nitrite. Nitrite accumulation is inhibited under frozen storage (WHO, 2003; EFSA, 2008).

A lot of studies have been conducted in European countries to determine the nitrite intake as a part of daily food. Based on national average food consumption data of France, average nitrite intake was 1.88 mg/day, 43% from vegetables; 28% from cured meats and 5 % from cereal products (Cornee et al., 1992). In a duplicate portion study in The Netherland, the highest recorded nitrite intake was 0.7 mg/day (Ellen et al., 1990).

In UK, the estimated mean dietary nitrite intake using the Total Diet Study ranged from 2.4-4.2 mg/day. However, this is recognized to be an overestimate as all samples containing undetectable levels of nitrite were assumed to contain nitrite at the limit of detection of the method used (1 mg/kg). In an earlier study in 1979, the estimated mean dietary nitrite concentration was 0.87 mg/day using methods with a lower limit of detection of 0.2 or 0.4 mg/kg (U.K. Ministry of Agriculture, Fisheries and Food, 1992).

Recent data on nitrite contents of vegetables has been summarized in Table 2. Data derived from Anonymous (1993); Ansorena and Merino (1992); Burt (1993; 1994); Cornee et al. (1992); Meah et al. (1994); Mortensen and Larsen (1989) U.K. Ministry of Agriculture, Fisheries and Food (1992).

Table 2. Nitrite levels in vegetables.

Vegetables	Nitrite content (mg/Kg)
Beans (Green)	ND
Beetroot	ND-8
Broccoli	2-4
Cabbage (Green)	ND-2
Carrot	ND-2
Celery (White)	ND-8
Cucumber	3
Potatoes	ND-19
Spinach	ND-26
Tomato	ND-4

ND: Not detected

Nitrate to nitrite ratio

The presence of nitrate and nitrite in vegetables and other food materials have been described in large number of scientific papers. The ratio of nitrate to nitrite has not been established till now as per our knowledge. Since nitrate as well as nitrite has significant impact on human health, it is important to review the ratio of nitrate to nitrite present in the vegetables. Nitrate and Nitrite content varies from place to place and from vegetable to vegetable. According to earlier surveys high values of nitrate anion concentration can be found in dill leaves (*Anethum graveolens*), lettuce leaves (*Lactuca sativa*), cabbage leaves (*Brassica oleracea* var. capitata), spinach leaves (*Spinacia oleracea*), amaranth (*Amaranthus* spp.), carrot root (*Daucus carota* subsp. sativus) and beetroot (*Beta vulgaris*). In the case of nitrite levels, most of the samples, if not all, fall under the detection or quantification limits (Croitoru et al., 2015).

Majority of the studies performed before 2001 has reported a low level of nitrate and nitrite in the vegetables (Yordanov et al., 2001) whereas the recently reported studies have shown higher values of nitrate and nitrite for the same vegetables (Santamaria, 2006). The higher values reported in the recent studies may be due to the development of sophisticated technology and new analytical methods with higher limit of detection (LOD) and limit of quantification (LOQ). A comprehensive data on nitrate to nitrite ratio has been presented in Table 3.

Table 3. Nitrate to nitrite ratio in vegetables.

S. No.	Vegetables	Nitrate (mg/kg)	Nitrite (mg/kg)	Nitrate: Nitrite ratio	Reference
1	Carrot (obtained in local market)	123.1	0.82	150: 1	Yordanov et al. (2001)
	Carrot (Home grown)	29.4	0.39	75 : 1	Yordanov et al. (2001)
	Carrot (Austria)	1500	-	-	Santamaria (2006)
2	Beetroot (Home grown)	38.9	0.45	86 : 1	Yordanov et al. (2001)
	Red Beetroot (Austria)	4500	-	-	Santamaria (2006)
	Red Beetroot (Germany)	3000	-	-	Santamaria (2006)
	Red Beetroot (Netherlands)	3500	-	-	Santamaria (2006)
3	Currants, gooseberries	36	-	-	Nabrzyski and Gaiewaska (1994)
4	Chinese kale	2340	83.8	28 : 1	Chou et al. (2003)
5	Organic non-heading Chinese cabbage	2860	41	70:1	Chou et.al. (2003)
6	Spinach	110	0.84	130:1	Jaworska and Kmiecik (1999)
	Spinach (obtained in local market)	860.1	7.44	115:1	Yordanov et al. (2001)
7	French bean (obtained in local market)	39.9	0.59	67 : 1	Yordanov et al. (2001)
	French bean (Home grown)	13.3	0.15	88 : 1	Yordanov et al. (2001)
	Organic green bean sprout	4410	85.1	52 : 1	Chou et al. (2003)
8	Lima Been (Frozen)	27	1.1	24 : 1	Siciliano et al. (1975)
9	Green bean	37.6	7.6	5 : 1	Afzali and Elahi (2014)
10	Onion (Winter)	14	0.3	47: 1	Siciliano et al. (1975)
	Green onion (obtained in local market)	30.1	0.30	100 :1	Yordanov et al. (2001)
	Green onion (Home grown)	11.2	-	-	Yordanov et al. (2001)
	Onion	12.20	6.09	2 : 1	Afzali and Elahi (2014)
11	Radish (obtained in local market)	737.2	5.93	124:1	Yordanov et al. (2001)
	Radish leaves	1521.13	-	-	Jana and Moktan (2013)
12	Paprika (obtained in local market)	38.1	0.48	79 : 1	Yordanov et al. (2001)
	Paprika (Home grown)	10.1	0.14	72 : 1	Yordanov et al. (2001)
13	Tomato (obtained in local market)	5.2	-	-	Yordanov et al. (2001)
	Tomatoes (Variety L2 Minidelicia)	112.5	0.3	375 :1	Simion et al. (2008)
	Tomato	4.3	0.3	14 : 1	Susin et al. (2006)
	Tomato	8.02	0.03	267 : 1	Andrei and Beceanu (2012)
	Tomato	7.82	1.81	4 :1	Rezaei et al. (2014)

S. No.	Vegetables	Nitrate (mg/kg)	Nitrite (mg/kg)	Nitrate: Nitrite ratio	Reference
14	Strawberry (obtained in local market)	17.5	0.23	76:1	Yordanov et al. (2001)
15	Potato (obtained in local market)	40.2	0.66	60:1	Yordanov et al. (2001)
	Potato (Home grown)	17.6	-	-	Yordanov et al. (2001)
	white potato tuber	12.67	0.06	208 : 1	Croitoru et al. (2015)
16	Chinese spinach	>3500	<1	3500:1	Chung et al. (2011)
17	Watermelon	26.61	5.5	4.84:1	Rezaei et al. (2014)
18	Cantaloupe	58.95	28.36	2.32 : 1	Rezaei et al. (2014)
19	Melon	33.64	7.65	4.40 :1	Rezaei et al. (2014)
20	Mushroom	63	0.8	78 : 1	Siciliano et al. (1975)
21	Cucumber	24	0.5	48 : 1	Siciliano et al. (1975)
		42.7	9.03	4.73 : 1	Rezaei et al. (2014)
22	Sprouts, soyabean (Winter)	63	0.8	78 : 1	Chung et al. (2003)
	Sprouts, soyabean (Summer)	52	0.9	58 :1	Chung et al. (2003)

Conclusion

Nitrates and nitrites present in vegetables have remarkable health benefits. The level of nitrates as well as nitrites varies significantly from vegetable to vegetable and demographic area. Green leafy vegetables and beetroot contain highest percentage of nitrates. The ratio of nitrates to nitrites varies from as low as (2:1) present in onion to as high as (3500:1) in Chinese spinach.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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How to cite this article:
Gupta, N. K., Jones, S., Kuruvilla, B. T., Benny, M., Antony, B., 2016. Nitrate to nitrite ratio in vegetables: A systematic review. Int. J. Curr. Res. Biosci. Plant Biol. 3(8), 74-80.
doi: http://dx.doi.org/10.20546/ijcrbp.2016.308.012