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Nitrate and nitrite content of vegetables: A review

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Abstract
Nitrogen is the most abundant element in the atmosphere and a crucial nutrient for the plant growth and development. Nitrate is a major form that plants absorb nitrogen. Vegetables can be considered as the main source of human nitrate intake. Although, nitrates are relatively low toxic, they can be reduced to nitrites within the human body and cause several adverse conditions such as methemoglobinemia. Nitrites also can be converted into N-nitroso compounds, which are known to be carcinogens. Therefore, several legislations have been established regarding the nitrate and nitrite content in food products. Several studies have been conducted to investigate the nitrate and nitrite contents in vegetables, and many of them have revealed that green leafy vegetables to contain high nitrate and nitrite levels. This review mainly focuses on the findings of the previously published studies regarding the nitrate and nitrite contents of vegetables and their potential health effects.

Keywords: nitrates, nitrites, N-nitroso compounds, vegetables, methemoglobinemia

Introduction
Nitrogen plays an important role on the life on earth, and the nitrogen cycle has a strong connection with the food chain and the properties of the food products [1]. It is an essential element for growth and development of plants and nitrate is the major form that plants absorb nitrogen [2]. Nitrogen fixation is the major way of adding nitrates into the soil and plants [3]. The equilibrium of nitrate concentration of vegetables depends on the rate at which nitrate is taken up by the plant and the activity of endogenous nitrate reductase. The nitrate and nitrite content of plants products depend on several factors including time of harvesting, addition of nitrogenous fertilizers, irradiation, type of growing, temperature, length of growth period and pedologic properties of soil and light [4, 5].
Inorganic nitrates and nitrites can be present in foods as naturally occurring compounds or as additives in processed foods [6]. The main sources of exposure to exogenous nitrates are vegetables and drinking water, while processed meats and animal food products are major source of nitrites [7]. Small amounts of these compounds may present in fish and dairy products [8]. More than 80-95% of nitrates are taken up by vegetables, especially green leafy vegetables such as lettuce, spinach and rocket [9, 10].
Concentrations of nitrates and nitrites in vegetables and other food products is an important quality indicator due to its influence on the human health [11]. Nitrates itself is relatively low in toxicity, but it is degraded into much more toxic nitrites within the human body [8]. Nitrite is ten times more toxic than nitrates [12]. Industrial revolution has caused an increase in concentration of nitrates and nitrites in foodstuffs, which now has become a global concern due to its harmful effects on environment and human health [13], and it is undesirable to have high levels of these compounds in foodstuffs [14]. Hence, many countries have paid their attention on the concentrations of nitrates and nitrites in different foods, especially in vegetables and baby foods [15]. Nitrates in vegetable have neither taste, nor smell, hence are analyzed through laboratory tests [16]. Therefore, many analytical techniques have been used for the determination of nitrates and nitrites in food stuffs. Some of them includes spectrophotometry, potentiometry, ion chromatography, polarography, capillary electrophoresis and High-performance liquid chromatography [16].

Nitrate and nitrite contents in vegetables
Unlike other nutrients, there is a lack of valid applicable and comprehensive database for nitrates and nitrites in foods. Hence, several studies were carried out in different countries regarding the nitrate and nitrite contents of food products, especially focusing vegetables [5, 11, 17, 18, 19, 20, 21] in order to develop a database with an aim of the precise assessment of nitrate/nitrite exposure in different populations, which aids in experiments regarding the health-related outcomes of dietary exposure to nitrate/nitrite [17].

Santamaria *et al*. [20] states that, the acceptable daily intake (ADI) for nitrate has been set as 0-3.65mg kg^{-1} body weight by both the Joint FAO/WHO Expert Committee on Food Additives and the Europian Communities' Scientific Committee for Food (SCF).

According to the studies, there is a wide variation of nitrate content of vegetables, ranging from less than 1mg $100g^{-1}$ to greater than 1000mg $100g^{-1}$ [9, 10]. A value of 10800mg kg^{-1} has been reported for a celery sample grown using hydrophonic technique [22]. The nitrate content of organically grown vegetables was higher than that of conventionally grown vegetables [23].

It has been reported that dark green leafy vegetables to contain extremely higher amounts of nitrates and nitrites, compared with other vegetables [3, 9, 24]. Spinach usually contain high nitrate concentrations, around 1000 ppm, and at some occasions, values exceeding 3000 ppm also have been reported. Lettuce also contain high nitrate concentrations, and the values up to 6000 ppm has been reported. This may be due to difficulties in removing soil adhered to them completely [22].

According to a study carried out in Greece, the nitrate and nitrite contents of vegetables that are commonly consumed in the country is relatively low, except some spinach, celery and lettuce, and among the vegetables, artichoke, cabbage, cauliflower, celery, kale and leek usually contain high nitrate contents, while asparagus, chicory and onions are lower in nitrates [25].

A survey conducted by the UK Ministry of Agriculture, Fisheries and Food (MAFF) revealed that lettuce, spinach, celery and beetroot had relatively high nitrate concentrations (greater than 1000 mg/kg), while potatoes, cabbage, spring greens had less (100-1000 mg/kg), and tomatoes had the lowest concentrations (less than 100 mg/kg) [8]. In Australia, spinach has the highest contribution for the dietary nitrate intake [7].

Abo Bakr *et al*. [14] has conducted a study to determine the levels of nitrates and nitrites in some vegetables commonly consumed in Egypt, using sixteen fresh vegetables under the different categories including leafy vegetables, pulses, root vegetables and others. The results of the study revealed that leafy vegetables to contain higher levels of nitrates and among them, the highest value was recorded in spinach, which was 5830 ppm. Other than these jew's mallow, roquette, chard and parsley contained high nitrate levels while the lowest levels were found in celery, lettuce and cabbage. As revealed by the study, the levels of nitrates recorded in pulses were in the range of 30-600 ppm, with the highest level in beans, which is lower compared to that of leafy vegetables. It was found that red and white raddish to have the highest nitrate content among the of the root vegetables. If further stated that the levels of nitrites in fresh leafy vegetables were higher compared to pulses and root vegetables [14].

A study [13] conducted using 13 vegetable varieties grown in south Tehran revealed Chinese Lettuce (*Lactuca sativa*) to contain the highest levels of nitrates and nitrites, which were 3637.63±60.09 and 6.37±0.50 ppm respectively. Another study [17], which was carried using several food commodities bought from Tehran found that radish, beetroot, tarragon, lettuce, mint and celery contained higher nitrate contents which were 6.3, 4.9, 4.2, 3.6, 2.8 and 2.6 mg g^{-1}, while the lowest average nitrate values were found in tomato, potato, garlic, corn, green beans, and carrot, which were 0.17, 0.38, 0.35, 0.28 and 0.47 mg g-1 respectively. The nitrite content of vegetables had a range of 0.21-0.74 mg 100 g^{-1} [17].

According to a study carried out in Italy, vegetables that belong to the families of *Chenopodiaceaceae*, *Brassicaceae*, *Apiaceae* and *Asteraceae* accumulate more nitrates, while *Convolvulaceae*, *Solonaceae* and *Liliaceae* contained the lowest levels of nitrates [20].

Zhong *et al*. [22] has found that, among nine vegetable types consumed in China, celery to contain the highest nitrate content (4600 mg kg^{-1}), and the lowest value was reported in tomato, which was 77.9 mg kg^{-1}. The study also found that eggplant and Chinese cabbage to contain the highest and lowest nitrite contents respectively.

A survey conducted by Chung *et al*. [5], to determine the nitrate and nitrite contents of vegetables grown in Korea during different seasons revealed that the mean nitrate contents to be higher in *Allium tuberosum* Roth (Crown daisy) (5150 mg kg^{-1}) and spinach (4259 mg kg^{-1}), while onion, soybean sprouts and green pepper contained less nitrates, which were 23, 56 and 76 mg kg^{-1} respectively, compared with other vegetables. The study also found that the average nitrite contents in various vegetables to be about 0.6 mg kg^{-1}, and the values were not significantly different among most vegetables. Most vegetables showed no significant variance in nitrate levels cultivated during the summer and winter harvests [5].

The nitrate and nitrite concentrations of vegetables and vegetable-based food products in Estonia was investigated by [26]. As revealed by the study, the highest mean values of nitrates were detected in dill, spinach, lettuce and beetroot, with the mean concentrations of 2936, 2508, 2167 and 1446 $mgkg^{-1}$ respectively, whereas tomato, onion and potato contained the lowest nitrate level which were 41, 55 and 94 mg kg^{-1} correspondingly. However, the recorded nitrite concentrations in vegetables were lower than 5 mg kg^{-1}.

Raczuk *et al*. [27] found that the nitrate concentrations of nine vegetable types, purchased from supermarkets in Poland to vary with a range of 10-4800 mg kg^{-1}, with the highest value in radish (2132 mg kg^{-1}), and the lowest in cucumber (32 mg kg^{-1}). The study also found that beetroot to contain the highest nitrite level (9.19 mg kg^{-1}), while carrots had the lowest (0.46 mg kg^{-1}) nitrite concentration.

Depending on the nitrate contents in fresh forms, vegetables can be grouped into five classes as follows [28].

1. Class I: Species with the contents mostly lower than 200 mg kg^{-1}
2. Class II: Species with the contents mostly lower than 500 mg kg^{-1}
3. Class III: Species with the contents mostly lower than 1000 mg kg^{-1}
4. Class IV: Species with the contents mostly lower than 2500 mg kg^{-1}
5. Class V: Species with the contents frequently higher than 2500 mg kg^{-1}

Ziarati *et al*. [13] discovered an association between the crop size and the nitrate/nitrite content. It states that the there is an inverse relationship between the nitrate content and crop size of onion, potatoes, beetroots, carrot, cabbage and lettuces. Large size onion, carrot, beetroot and potato had significantly lower nitrate levels than small size. However, green leafy vegetables showed a positive correlation between the size and the nitrate content [13].

Generally, the nitrite levels in vegetables is much lower than that of nitrates [29]. Fresh, undamaged and well-stored vegetables contain extremely low nitrate contents as the nitrite reductase activity rate is in equilibrium with one of the nitrate

reductase enzymes under proper storage conditions. Bacterial growth can contribute to increasing accumulation of high nitrite levels [30]. Failure of endogenous nitrite- reductase to match nitrate-reductase activity may also increase the nitrite levels in vegetables [31]. Generally, vegetables that accumulate high nitrate levels may contain higher amounts of nitrite [25].

Nitrate content decreases during storage under ambient temperature due to its conversion to nitrites. Under refrigerated conditions the nitrite accumulation tends to be inhibited, but may take place. The nitrite accumulation is inhibited under frozen storage [16, 30]. Therefore, there can be different contributions of both nitrate and nitrite in commercially available vegetable samples [32].

A summary of the nitrate and nitrite contents of some vegetables recorded in several studies is depicted in the table 1.

Effects of cooking and storage conditions on nitrate contents of vegetables

According to Prasad and Chetty [33] the nitrate contents of root vegetables decreased on boiling. This may be due to the leaching of nitrates into cooking water as they are easily water soluble. The nitrate values remained relatively constant after baking but increased after frying in soya bean oil. This increase of nitrate content may be due to the loss of moisture content during frying and, the small sample size, high amount of oil used in frying and the presence of nitrogenous spices in soya bean oil. [33, 34].

Santamaria *et al*. [20] states that at least 50% of accumulated nitrates can be removed by cooking vegetables in water with low nitrate concentrations. Cooking decreases the nitrate levels in vegetables than do blanching [35].

Nitrite accumulation in vegetables are inhibited under frozen storage due to the inactivation of endogenous nitrate reductase [36]. The nitrite content of frozen spinach was found to be lower than that of fresh ones [37].

Abo Bakr *et al*. [14] revealed that cooking to have an effect of lowering nitrate content of vegetables, and no nitrites were formed during cooking. Leafy vegetables showed the highest nitrate loss during cooking. The study also showed that the nitrate and nitrite contents of potatoes were heat stable during cooking, but losses occurred due to leaching from potato tissues into cooking water. Ziarati *et al*. [13] states that the contents of nitrite do not change during cooking for most vegetables, but only in potato and onion were reduced with boiling.

Canned foods contain less amount of nitrates compared with the frozen products, and this may be due to the distribution of nitrate between the solid and the liquid portion [37].

Aworh [38] has found that spinach samples stored in modified atmosphere had increased nitrate to nitrite conversion than the plants stored in normal atmosphere. The conversion nitrate to nitrite increased also with the increasing temperature [39].

Removing high nitrate containing parts such as peeling potatoes and beetroot and removing the midrib of lettuce and spinach decrease the nitrate content [40]. Pureeing of high nitrate containing vegetables may cause excessive formation of nitrite due to the release of endogenous nitrate reductase [41].

Prolonged storage of opened, thawed, cooked, or uncooked vegetables or their storage under improper conditions may lead to conversion of part of their nitrate to nitrite [42]. However, fresh, uninjured and well-stored vegetables are extremely low in nitrites [30].

Nitrate metabolism in plants

Nitrogen level in the plant is the most frequent factor that affect the crop productivity [2]. Nitrogen is an essential nutrient for the plants and animals, as it is required in numerous essential components such as proteins, DNA, RNA, vitamins, hormones and enzymes. Plants absorb environmental nitrogen in the forms of nitrates and ammonia [33]. Ammonium transporters are responsible for the absorption of ammonium ions into the plants while, nitrates are taken up across the plasma membrane by several nitrate transporters (NRTs). This is the first step of nitrate assimilation and a major step in controlling it [43, 44, 45]. Nitrate is accumulated in plant vacuoles. The major functions are osmotic potential regulation (when the concentrations of organic acids and soluble sugars decline), acting as a signal molecule for the activation of acid metabolism, inhibition of starch synthesis and for governing root and shoot balance [46, 47].

According to the findings of some studies, high nitrate accumulation in plants leads to nitrite production within the cytoplasm, which is then converted into nitric oxide (NO). This NO together with O_2^- could be rapidly formed into peroxynitrite ($ONOO^-$), by nitrate reductase (NR). This compound is highly toxic to the plant [48, 49].

The nitrite is transported to chloroplast by a nitrite transporter (NRT1) and it is then reduced to ammonia by nitrite reductase (NiR). Genetic factors, substrate induction and the supply of electrons from NADPH are the major factors that influence the rate of reduction of nitrate by endogenous nitrate reductase [31].

Several factors may be responsible for reduction of nitrates including, the presence of bacteria or enzyme nitrate reductase, and in contact with metals. At acidic pH, nitrite is unstable and can disproportionate to yield nitrate and nitrogen oxide and/or react with food components including amines, phenols and thiols [34].

Factors affecting nitrate and nitrite contents in vegetables

Nitrate and nitrite content of vegetables were extremely variable and varied from country to country and region to region, as revealed by several studies [10, 26. 32]. Large variations of nitrate contents between the different samples of the same vegetable have been reported [16]. These variations may be attributed mainly to environmental, genetic and nutritional factors [50]. Cardenas-Navarro *et al*. [51] stated that the accumulation of nitrates in plant tissues may be due to an imbalance between nitrate uptake and its assimilation.

Bahdoran *et al*. [17] have reported an extensive variation of nitrate in vegetables in different countries and regions, with a range of <1 to >1000 milligrams per hundred grams [9, 10]. Factors including cultivar type, composition of soil, light intensity, air temperature and moisture, growth density, duration of growth period, harvesting time, storage time, edible plant portion and use of nitrogen fertilizers and legislations may be accountable for these variations [7, 10, 26, 32]. Out of these factors, nitrogen fertilization and light intensity has the major influence on the nitrate content of vegetables [52, 53]. Nitrate levels in plant parts are also very sensitive to changes in Nitrogen supply [25], and the capacities to accumulate nitrate depends on the different locations of the nitrate reductase activity and the different degree of nitrate absorption and transfer in the plants [53].

The nitrate content varies between the different portions of the plant and it can be listed in decreasing order as follows [20].

Petiole> leaf> root> stem> inflorescence> tuber> bulb.

Health benefits of nitrates and nitrites

It has been reported some therapeutic properties of nitrites and nitrites, in several pathological conditions including cardiovascular diseases, diabetes, hypertension, metabolic syndromes and insulin resistance [54, 55, 56, 57, 58, 59].

Recent researches have shown the beneficial effects of nitrate on human health based on the hypothesis that nitric oxide formed in the stomach by dietary nitrates has an antimicrobial effect on pathogenic microorganisms living in the guts [53]. Dykhuizen *et al*. [60] states that the high plasma nitrate levels in patients suffering from infective gastroenteritis may protect against the fecal-oral route of reinfection via increased generation of salivary nitrite.

According to Jones [61], nitrate especially in form of beetroot juice may be an ergogenic supplement and improve exercise performance and capacity.

Several studies have found that nitrate metabolites to have important physiological roles in the body such as vasodilation, blood pressure regulation, inhibition of endothelial inflammatory cell recruitment, and platelet aggregation. [10, 62, 63].

Negative health impacts of nitrates and nitrites

Approximately 5% of all dietary nitrates are reduced to nitrites in saliva and the gastrointestinal tract [64]. Increased dietary exposure to nitrates and nitrites is becoming an important public health issue [40, 65, 66], due to the formation of nitrosoamines, which leads to some acute and chronic toxicities such as methemoglobinemia, throid desorders and carcinogenesis [65, 67, 68].

Methemoglobinemia is a condition where reduced iron (Fe^{2+}) in haemoglobin is oxidized to $Fe^{3+,}$ producing methaemoglobin. This results in impairment of oxygen delivery to human tissues [69, 70].

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

Vegetables are an important part of the diet of infants, and excessive nitrites in the diet may cause infantile methemoglobinemia due their low stomach acidity [71, 72, 73]. Infants under 3 months and young children are most susceptible for this condition due to lower stomach acidity, which favours the growth of nitrate reduction bacteria, presence of fetal hemoglobin, which is readily oxidized by nitrite and lower levels of NADH-dependent methemoglobin reductase [40, 53]. This is also known as blue baby syndrome as when the methemoglobin reaches 10% of normal Hb level, blue discolouration of the skin due to the presence of deoxygenated blood occur [69, 74]. Due to the induction of methaemoglobin reductase during the physiological post-weaning period, children and adults are less susceptible to methemoglobinemia [62, 70].

Nitrates, when reduced to nitrites, may react with amines or amides and produce N-nitroso compounds [3, 70]. N-nitroso compounds have shown carcinogenic effects in more than 40 animal species, including human [34, 75]. Several experiments have been performed in order to determine the carcinogenicity of about 300 N-nitroso compounds (NOCs). Out of them, 85% of the 209 nitrosamines and 92% of the 86 nitrosamides have been shown to be carcinogenic in a variety of species [8]. Although the nitrtite intake is usually lower than the ADI, presence of nitrites in both foods and body is a huge problem due to the formation of nitrosoamines [36].

Van Maanen *et al*. [76] has shown that nitrate can interfere with iodine uptake by the thyroid, resulting in hypertrophy of the thyroid. Some studies have found an increased risk of thyroid cancers with higher nitrate/nitrite intake [77, 78]. The excessive production of nitric oxide within the body can lead to functional abnormalities associated with gastro-esophagal reflux disease [79]. High nitrate ingestion is associated with increased risk of cancers in urinary bladder [75], esophagus, nasopharynx and prostate [80], colon and other gastrointestinal regions [81, 82], and oral cancers [83]. It can also leads to Alzheimer's disease, vascular dementia of Biswanger type or multiple small infarct type [84], multiple sclerosis [85], spontaneous abortion or congenital defects [65], anencephaly [86], non-Hodgkin's lymphoma [87] and cardiovascular disorders [88]. High nitrate ingestion also has an association with recurrent diarrhea in children and recurrent stomatitis [89, 90].

Nitrites may decrease the nutritional value of consumed vegetables as it affects carotenoid, vitamin A and the B group vitamins degradation [27].

The increasing concern on nitrated toxicity has directed a number of countries to lay down maximum allowable concentrations of nitrates in vegetables [33]. It has been reported that some components in vegetables, such as ascorbic acid, phenols and etc. has the potential to inhibit the toxic effects of nitrites [8, 74]. Fruits and vegetables which are rich in ascorbic acid, tocopherols, carotenoids and flavonoids are able to inhibit the formation of N-nitro compounds [8, 91].

Table 1: Comparison of nitrate and nitrite content of commonly consumed vegetables recorded in studies carried out in different countries

Food Crop	Chung *et al*., (2003)/ Korea		Ziarati *et al*., (2014)/ Iran		Raczuk *et al*., (2014)/ Poland		Bahdoran *et al*., (2016)/ Iran	
	Nitrates (mg kg^{-1})	Nitrites (mg kg^{-1})	Nitrates (mg kg^{-1})	Nitrites (mg kg^{-1})	Nitrates (mg kg^{-1})	Nitrites (mg kg^{-1})	Nitrates (mg g^{-1})	Nitrites (mg $100g^{-1}$)
Chines cabbage	1740	1.1	-	-	-	-	-	-
Cabbage	725	0.4	1272.89±39.87	1.43±0.48	436	0.88	2.0±1.5	0.54±0.24
Cauliflower	-	-	195.29±45.37	0.96±0.08	-	-	1.0±0.92	0.39±0.23
Lettuce	2430	0.6	3637.63±60.09	6.37±0.50	1725	1.64	3.6±2.3	0.54±0.31
Spinach	4259	1.0	3440.73±15.66	6.18±0.87	-	-	1.8±1.2	0.43±0.29
Leek	-	-	-	-	-	-	1.8±0.96	0.61±0.28
Soy bean sprouts	56	0.9	-	-	-	-	-	-
Onion	23	0.3	999.96±17.19	4.04±0.62	-	-	0.61±0.79	0.49±0.30
Green onion	436	0.3	-	-	-	-	-	-
Squish pumpkin	639	0.6	-	-	-	-	-	-
Cucumber	212	0.3	-	-	32	0.50	0.88±0.70	0.57±0.31
Potato	452	0.5	520.69±27.33	0.88±0.16	54	0.50	0.38±0.17	0.49±0.33
Carrot	316	0.4	480.07±0.15	1.01±0.07	82.2	0.46	0.50±0.30	0.48±0.28
Beet root	-	-	3045.54±55.50	1.02±0.22	1306	9.19	4.9±1.3	0.56±0.37
Radish	1878	0.8	-	-	2132	1.90	6.3±3.2	0.49±0.40

Garlic	124	0.2	-	-	-	-	0.35±0.06	0.21±0.08
Green pepper	76	0.4	-	-	-	-	-	-
Allium tuberosum roth	5150	0.9	-	-	-	-	-	-
Tomato	-	-	-	-	35	0.82	0.17±0.12	0.55±0.26
Egg plant	-	-	247.16±9.69	0.96±0.08	-	-	1.1±1.1	0.72±0.28
Green beans	-	-	-	-	-	-	0.47±0.37	0.38±0.34

***Sources*:** 5, 13, 17, 27

Conclusion

Nitrates and nitrites can be ingested by human mostly, through vegetables. The levels of nitrates and nitrites in vegetables have become a major public concern due to their negative health impacts. Therefore, several studies have conducted in different countries, in order to determine the levels if nitrates and nitrites in vegetables and, most of them have recorded higher levels in leafy vegetables. Hence, some countries and institutions have established regulations for the control of nitrate contaminations of vegetables.

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