# AGRICULTURAL PRACTICES TO MINIMIZE NITRATE ACCUMULATION IN EDIBLE PARTS OF CROP PLANTS

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## Summary

The growth of crops depends heavily on the availability of nitrogen, which is taken up by plants predominantly in the form of nitrate, which is reduced for most species in the leaves to ammonium to form amino acids and proteins. Humans and animals rely on the protein built by plants. Thus, the use of mineral N fertilizers is essential to feed the world population, but it is also associated with risks such as increased nitrate concentration in the edible plant parts. The toxicity of nitrate is low, but it is reduced by microorganisms to nitrite, which is known to cause methemoglobinemia in infants and with amines to form nitrosamines, which are known to induce cancer. Up to 70% of the nitrate taken up by humans is derived from vegetables.

The most significant tool for the farmer to control nitrate concentration in the crop is an appropriate N fertilization rate, which means the avoidance of over-fertilization or even the acceptance of yield reductions in order to produce a crop containing low nitrate

concentrations, as may be necessary for the processing of dietary food. Further approaches to controlling the nitrate concentration in plants are the partial supply of N in the form of ammonium, including an effective nitrification inhibitor, or the supply of $Cl^-$. In protected cultivation, soil-less culture is an additional opportunity. Produce from organic farming potentially contains less nitrate. This is the result of low nitrogen availability in such production systems, which frequently limits yield, but does not depend on the origin, organic or mineral, of the N source. Apart from management practices, variety selection is a powerful tool to reduce nitrate content in the produce. Furthermore, the nitrate concentration may be reduced in spinach by excluding petioles in the harvested crop. Outer leaves and petioles of head lettuce should be avoided. To meet the specific low threshold values for dietary food production, vegetables should be planted early in the season to be harvested under high global radiation conditions.

## 1. Introduction

The mineral nutrient nitrogen is needed in large amounts by plants because it is a constituent of macromolecules, such as protein. However, only some plants living in association with $N_2$-fixing bacteria can use the di-nitrogen gas contained in the air. The majority of plants rely on ammonium and nitrate that originate from the decomposition of organic matter and are taken up from the soil, and the availability of N often limits plant growth. The breakthrough in the improvement of N nutrition and plant production was the invention of technical ammonia synthesis in 1913 by Haber and Bosch. The main forms contained in fertilizers are nitrate, ammonium, and urea, but plants predominantly absorb nitrate, since fertilized ammonium and urea is microbially converted to nitrate in the soil, a process called nitrification. The N supplied from the soil by mineralization of organic matter is also subjected to this process. The inorganic N forms, nitrate and ammonium, in the soil can be actively absorbed by plants.

The nitrate taken up is reduced by the "nitrate reductase" and "nitrite reductase" enzymes to ammonia, which is fixed into glutamate to produce glutamine. The glutamine provides the amino group for synthesis of amino acids, which are constituents of proteins. The nutrition of man and animals relies on protein synthesized by plants. Human beings need about 50g of protein per day, which is equivalent to 8g of N. Thus, the ammonia synthesis invented by Haber and Bosch provides an important basis for feeding the world population. However, the increased use of N for plant production, apart from its beneficial effects, creates serious problems. The nitrate anion, the inorganic N form mainly occurring in the soil, is not bound to the solid phase in the soil and it is very mobile in the soil solution; thus it can be leached easily into the groundwater or lost via drainage, leading to the eutrophication of rivers, lakes and coastal areas. Furthermore, nitrate may be lost via denitrification, a microbial process that is induced under oxygen deficiency in the soil, which is often due to precipitation and irrigation. The products of this process are $N_2$, $N_2O$, and NO. $N_2O$ is an important greenhouse gas in the troposphere leading to global warming, and it is involved in the depletion of the ozone in the stratosphere.

The availability of nitrogen also affects the quality of the produce. These effects might be positive, as in the case of the baking quality of wheat, but also negative regarding the nitrate content of the produce. In summary, N fertilization is essential for feeding humans, but the increased use of freely available nitrogen also creates problems. One of these problems, the nitrate content in plant food for human consumption, will be discussed in this contribution.

## 2. Hazards of Nitrate to Health

About 70% of the nitrate that is absorbed by humans originates from vegetables and 20% from drinking water. The toxicity of nitrate itself is relatively low and the fatal adult dose is reported to be as high as 7-35g. This is about 100-times higher than the acceptable daily intake (ADI) via food and beverages, which is 255mg nitrate day$^{-1}$ for a person of 70kg (WHO). The risk is due to nitrite, which is formed by reduction from nitrate. This process may happen during the storage of fresh or cooked food as well as in the human alimentary canal. Nitrate reduction in fresh or cooked food is caused by microorganisms, which use nitrate for respiration under anaerobic conditions. A closer look will be taken at the process of nitrate reduction in man.

Nitrate taken in with food passes through the stomach and is adsorbed in the small intestine, leading to a peak of nitrate concentration in the blood plasma two to three hours after oral intake. The elimination half-time is remarkably long, 10.8 – 12.7 hours. The course of the nitrate concentration in the blood plasma is paralleled by both an increase in nitrate and nitrite concentration in the saliva. Nitrite is formed by microorganisms of the mouth flora and passes with saliva to the stomach. However it is thought that nitrate reduction may occur in the large intestine as well, since pH is > 5 and the microorganism density is high. A significant increase in nitrite concentration in saliva occurs after absorption of more than 50mg nitrate. This means that the same amount of nitrate taken up with one meal leads to a higher amount of nitrite compared to an absorption distributed over several meals. Most of the nitrate taken in is excreted in the urine, 10-20% is recycled via saliva and 3-6% is reduced to nitrite.

### 2.1. Methemoglobinemia

Methemeglobinemia is induced by nitrite which, oxidises hemoglobin to methemoglobin; the latter is not capable of transporting oxygen:

$$\underset{\text{Hemoglobin}}{2\ \text{Hb}\ (\text{Fe}^{\text{II}}) - \text{O}_2} + \text{NO}_2^- + \text{H}_2\text{O} \rightarrow \underset{\text{Methemoglobin}}{2\ \text{Hb}\ (\text{Fe}^{\text{III}}) - \text{OH}} + \text{NO}_3^- + \text{O}_2$$

At a level of 10-20% methemoglobin, a decreased oxygen supply to the tissue may be expected, and a level of more than 70% may lead to death. The occurrence of methemeglobinemia is restricted to infants not older than three months for the following reasons: (I) they have up to 80% fetal hemoglobin, which is more susceptible to oxidation, (II) the enzymatic reduction rate of methemoglobin to hemoglobin is lower compared to adults (III) acid production in the stomach is low and thus acidity allows growth of bacteria, increasing the potential for nitrite formation.

Methemoglobinemia of infants was described in 1945 as result of nitrate ingestion via drinking water. It is also called “blue-baby” syndrome or cyanosis, and sometimes also well-water methemoglobinemia since it was always related to the use of water from a well. This was the main reason for reducing the threshold value for nitrate in the drinking water in the European Community, as well as in the United States of America, to 50 mg $l^{-1}$. However, cases of methemoglobonemia have also occurred after the ingestion of spinach, which is known to be a vegetable with high nitrate content that may be reduced to nitrite during storage of the fresh or cooked product. This led to the recommendation not to store cooked spinach. The incidence of methemoglobinemia has become rare after the discovery of the causes and the introduction of precautions to avoid the hazards. In the United Kingdom, the last death occurred in 1950, and the last case was confirmed in 1972.

Microbial flora in the rumen readily converts nitrate to nitrite, making ruminants particularly susceptible to such toxic effects of nitrite.

### 2.2. Cancer

In the stomach, nitrite may form nitrosamines by nitroation with amines, which are known to cause cancer:

(1) $2\ HNO_2 \rightarrow N_2O_3 + H_2O$

(2) $$\left.\begin{array}{l} R_1 \\ R_2 \end{array}\right> NH + N_2O_3 \rightarrow \left.\begin{array}{l} R_1 \\ R_2 \end{array}\right> N - N - O + HNO_2$$

Two molecules of nitric acid form $N_2O_3$, which reacts with amines. The example given above shows the reaction with a di-amine to form nitrosamine. The concentration of many nitrosamines is exponentially related to the nitrite concentration. Only the non-protonated amine can be nitrosated. The quantity of non-protonated amine in the acid stomach content depends on the pK value of the amine. Furthermore, nitrosation is blocked by large amounts of vitamin C.

Nitrosamines, as such, do not induce cancer, but the electrophile transformation products are known to react with DNA, leading to chromosome cracks. The carcinogenic potential of nitrosamines depends on their lipophility; it has been shown that tumours in rats were induced by just 6 $\mu g\ kg^{-1}$ of dimethylnitrosamine, but more than 20 000 $\mu g\ kg^{-1}$ of nitrosoproline. The organs where tumours are induced depend on the nitrosamine species, dose, and application method. There is evidence that nitrosamines are also potentially carcinogenic in humans, since this has been shown for more than 20 animal species, including monkeys.

However, it is not certain that endogenously-formed amines will reach tumor-causing levels. Nitrosamines are contained in salted foods, such as meat, fish, sausages, cheese and pickled vegetables. However, cigarette smoke may contribute even more nitrosamines to daily intake than food. A case study in Thailand suggests that consumption of food such as fermented fish that contains large amounts of nitrosamine play an important role in carcinogenesis associated with the infestation of the liver fluke parasite *Opisthorchis viverrinae*.

Although it has not been proven that consumption of nitrate-rich vegetable food leads to significant endogenous nitrosamine production, for preventive reasons, the nitrate load should be kept as low as possible. This will be discussed in the following paragraphs.

## 2.3. Threshold Values

For drinking water, the threshold value of 50 mg $NO_3$ $l^{-1}$ has been established in countries of the European Union, as well as in the United States of America, since case studies showed that methemoglobinemia increased significantly with nitrate concentrations higher than 100 mg $l^{-1}$ in well water.

In 1997, the European Union introduced threshold values for some vegetables, which are summarized in Table 1. Values vary between species and season, since the nitrate content depends strongly on both factors. Furthermore, a threshold level of 250 ppm nitrate in ready to eat infant food and all dietary food was introduced in Germany.

| Species | Harvest Season | Threshold value mg $NO_3$ kg f.m.$^{-1}$ |
|---|---|---|
| Spinach, fresh | April – October<br>November – March | 2500<br>3000 |
| Spinach, processed and deep frozen | | 2000 |
| Lettuce, greenhouse | April – September<br>October – March | 3500<br>4500 |
| Lettuce, field | May - August | 2500 |

Table 1. Nitrate threshold values in vegetable crops introduced by the European Union in 1997
(Anonymous (1997): European Community regulation No. 194/97).

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**Biographical Sketch**

**Manfred K. Schenk** was born on 29 March 1947. He studied Horticulture at the Faculty of Horticulture at the University of Hanover, Germany, from 1967 to 1971. After completing his studies, he worked for one year as assistant to the President of the University of Hanover. During the following years, he worked on his Ph.D. under the guidance of Prof. Dr. J. Wehrmann. He earned his Ph.D. degree in Plant Nutrition in 1977 from the Faculty of Horticulture. In 1978, he was a post doctoral fellow in the laboratory of Prof. Dr. S.A. Barber, Purdue University, West-Lafayette, USA. In 1979, he was appointed head of the Horticultural Research Station of the Agricultural Chamber of Westfalia-Lippe in Münster. Since 1984, he has been Professor of Plant Nutrition at the University of Hanover.

His scientific work in plant nutrition focuses on mineral nutrient acquisition by roots from soil and substrate. A further research field is nutrient dynamics in the soil/substrate - plant system, including the forecasting of fertilizer requirements and heavy metal transfer from soil to plant. Another research area is the nutrient efficiency of plant species and genotypes. Most of the research is conducted on the nutrients nitrogen and phosphorus, whereas the heavy metal primarily investigated is cadmium. Research is conducted on both vegetable crops, as well as ornamental plants grown in protected cultivation or in the field.

From 1991 to 1995, he was Dean of the Faculty of Horticulture, and from 1995 to 1999, was a member of the Senate of the University of Hanover. He served the German Society of Horticultural Science from 1990 to 1996 as Vice President, and from 1996 to 2002 as President. Since 1994, he has been a member of the Council of the International Society of Horticulture.