STUDY ON THE STORAGE CONDITION INFLUENCE UPON THE NITRITE CONTENT IN VEGETABLES

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Abstract

The paper presents the nitrite sources, the ways by which the nitrite gets into the human body and its negative effects on health. Nitrites also may derive from the nitrite reduction in the presence of a reducing flora. The nitrate presence in products of vegetable origin is generally the consequence of soil over-fertilization with nitrous fertilizers. One of the most important nitrite sources are vegetables, consumed by all categories of age. By determining the nitrite content in the most consumed vegetables, spinach and salad, it has been demonstrated that nitrite may also be found in the lowest proportion in fresh vegetables, and its content increases when keeping the latter ones in the refrigerator. As the nitrite content increases over 5-8 times at refrigeration, during 3 -7 days, vegetables should be consumed fresh only when they have benefic effects upon health.

Résumé

Ce travail présente les sources de nitrite, les moyens par lesquels celui-ci arrive dans l'organisme humain et ses effets négatifs sur la santé. Les nitrites peuvent résulter aussi de la réduction des nitrites en présence d'une flore réductrice. La présence des nitrates dans les produits d'origine végétale est en général la conséquence de la sur-fertilisation du sol avec des engrais azoteux.

Une des sources de nitrite les plus importantes sont les légumes, consommées par toutes les catégories d'âge. Par la détermination du contenu de nitrite chez les légumes les plus consommées, l'épinard et la salade, on a démontré que le nitrite se trouve dans la plus petite proportion dans les légumes fraîches, et son contenu augmente pendant leur réfrigération. Lorsque le contenu de nitrites augmente de 5-8 fois pendant la réfrigération pour 3-7 jours, il faut les consommer seulement fraîches quand elles ont des effets bénéfiques pour la santé.

Rezumat

In lucrare sunt prezentate sursele de azotit, căile prin care acesta ajunge in organismul uman şi efectele negative ale acestuia asupra sănătăţii. Nitriţii pot proveni şi din reducerea nitraţilor în prezenţa unei flore reducătoare. Prezenta azotaţilor în produse de origine vegetală este în general consecinţa suprafertilizării solului cu îngrăşăminte azotoase
Una dintre cele mai importante surse de nitrit sunt legumele, consumate de toate categoriile de virsta Prin determinarea continutului de azotit la cele mai consumate legume, spanacul si salata, s-a demonstrat ca azotitul se găseşte in cea mai mică proporţie în legumele proaspete, iar conţinutul său creşte la păstrarea acestora in stare refrigerată. Deoarece conţinutul de azotiţi creşte la refrigerare, in timp de 3- 7 zile de peste 5- 8 ori, legumele trebuie consumate numai in stare proaspătă cind au efecte benefice asupra sănătaţii.

Introduction

The use of nitrate fertilizers on larger and larger areas and for very different cultures has determined at the same time the necessity to research the influence of nitrogen compounds upon the agricultural produces quality as well as the human body and animal reaction towards produces obtained from treated plots of land. Since the soil is reciprocally interdependent on the hydrosphere, the latter one will also have high nitrate content resulted from soil fertilization. Another source of water polluting consists of the waters used in the animal breeding and poultry farms, the waste waters resulted from the domestic and animal residues. The nitrates from water derive from the ammonia incomplete oxidation in the presence of denitrifying bacteria. The nitrites represent a more advanced stage in the decomposition processes of the substances containing nitrogen and consequently their presence or the normal concentration increase in water shows an older pollution of days-weeks. Nitrites may also derive from the nitrate reduction in the presence of a reducing flora and higher environment temperature. The nitrate presence in produces of vegetable origin is generally the consequence of soil over fertilization with nitrogenous fertilizers such as: Na nitrate (saltpetre of Chile), Ca nitrate (saltpetre of Norway), K nitrate, ammonium nitrate (saltpetre of Montana or Leona),

urea, ammonium sulphate or chloride, ammonia, Ca cynamid. Some pesticides used in the agriculture may also lead to the increase of nitrate content in plants. Therefore, when nitrates are present in available excess for plants, they are not used anymore for amino-acid and protein synthesis, but they are being accumulated in plants as such. The nitrate accumulation in plants depends on meteorological conditions and soil pH which influence the solubility and assimilation of oligominerals by the plant, oligominerals (manganese, molybdenum, copper, magnesium) which favour the nitrate accumulation in plants or their reduction to nitrites.

The produces cultivated on fertilized plots, as it results from the determination made, contain always highly increased quantities of nitrates than the ones cultivated on non-fertilized soils

Table 1: Nitrate Content in Different Produces, NO^{-3}mg/kg (average values)

Current Nr.	Produces	Non-fertilized soil NO^{-3}mg/kg (average values)	Fertilized soil NO^{-3}mg/kg (average values)
1.	Onion	-	1186
2.	Potatoes	40	342
3.	Carrots	65	460
4.	Radishes	960	2160
5.	Cucumbers	153	527
6.	Cabbage	15	151
7.	Salad	318	3547
8.	Fresh spinach	-	2215

Mention must be made about the fact that nitrates are present in cereals (wheat, barley, oat, rye, maize) too, where it was made evident up to 57mg nitrates per kg produce. Fruit contain reduced quantities of nitrates (<10 mg per kg), except bananas and strawberries wherein the nitrate content varies between 24-140 mg/kg.

The injurious/noxious effects due to the dosage increase of nitrate substances in fodder plants become more prominent if other factors intervene /interfere such as:

- Cold which slows down significantly the biosynthesis process , except the radicle absorption;
- Excessive heat (drought), because of which the water in soil necessary to biosynthesis is evaporated;
- Cloudy and dark weather which diminishes the biosynthesis process;
- Rainy weather after drought, when a lack of balance between absorption and biosynthesis takes place;
- The molybdenum microelement absence in soil which participates in reducing the nitric ion;
- Deficiency of carbohydrates in plants which participate in biosynthesis.

All these factors lead to the deficit metabolizing of nitrates got into the plant by radicle absorption and so, to their accumulation. The plant which keep their balance between the absorbed water and that evaporated through leaf-pores under the form of drops are destitute of nitrates as it happens with the vivid graminaceae. In wheat, 50% of the absorbed nitrates are not turned into protein, but carried together with sap to aerial parts. The highest concentration of nitrates is found in the stem.

Nitrates and nitrites may also be found in produces of animal origin: in milk, deriving from fodders; in meat, from intentional addition.

Nitrites Action Upon Human Body

The level of nitrates and nitrites present in the human body will depend on: the ingested quantity of food products containing nitrates/nitrites and the endogenous production of nitrates/nitrites. Important quantities of nitrites (and fewer nitrates) are formed endogenously in the gastro- intestinal tract (oral cavity and intestines).

Table 2: Human Body Charge with Nitrite

Nitrite source	mg/day
Nitrite brought by food products	3
Nitrite produced in the oral cavity by microorganisms and carried by saliva	15(10-20)
Nitrite produced in intestines by microorganisms	90(60-100)
Total	108 mg/day

The nitrate ingested together with food gets into the stomach and intestines, wherein is more or less turned into nitrite, depending on the individual's health state. Thus, in the case of individuals having deficiency in gastric secretion, the stomach is populated with bacteria and thus the nitrate is mostly turned into nitrite (forming 100-100 mg nitrite/kg).

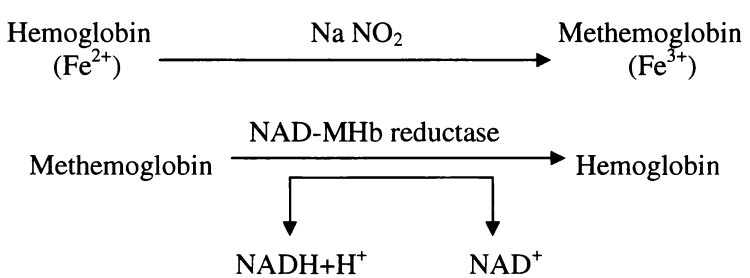
This nitrite formed in the stomach is a risk yield in causing stomach cancer. A healthy individual has in his stomach very small quantities of nitrate (< 1 mg/kg). Under conditions of the urinary bladder bacterial infection, the nitrate is turned into nitrite and at the level of this organ, the respective nitrite is a risk yield in the urinary bladder cancer. The absorption of the untransformed nitrate takes place in the small intestine which partially gets into blood and in 24-48 hours is excreted from the blood plasma through kidneys. Another part of nitrate gets to the salivary glands and from here, together with saliva in the oral cavity. In the oral cavity, the nitrate is reduced to nitrite by the herein present denitrifying micro-flora. The maximum quantity of nitrate brought by saliva in the oral cavity (100-1000 mg/kg) may be found three hours after food ingestion.

The nitrite ingested together with food, the one produced in the oral cavity or in the aerial part of the intestinal tract by the heterotrophic nitrifying bacteria, from ammonia or other organic substances containing nitrogen, is partially re-absorbed in blood, converting the hemoglobin into methemoglobin, which is not capable of transporting the oxygen, resulting in anoxia, sometimes serious enough to cause the individual's death by asphyxiation.

Nitrites are extremely toxic having the following actions:

- Cause methemoglobinemia by reacting with hemoglobin, the degree of methemoglobinemia depending on the quantity of nitrites passed into blood so on the quantities nitrates/nitrites ingested together with food and endogenously made up.

  The methemoglobin level in blood is 1-2% of hemoglobin total, the common reduction process of methemoglobin being mainly carried out by an enzyme which as co-factor $NADH+H^+$(NAD- methemoglobin reductase) which may be found in erythrocytes.



  If the methemoglobin degree does not go beyond 50% and the nitrite supply ceases, then the methemoglobin may reduced to hemoglobin by NAD- methemoglobin-reductase. Death occurs at a higher degree of methemoglobinemia than 50%. The methemoglobinemia "acquired" as a result of the nitrite action in the case of suckling is by far more dangerous as the baby has deficiency in NAD- methemoglobin-reductase or in its co-enzyme NAD. The

new-born children's hemoglobin is in proportion of 60 – 80 % of foetal type which oxidizes easier as compared to the hemoglobin in grown-ups 'blood.
Methemoglobinemia caused by nitrite incriminated food is occasional, being generated at children who have consumed smashed spinach, carrots containing high quantities of nitrates/nitrites. The ascorbic acid from vegetable produces has a protecting effect against nitrates/nitrites.

- Cause the lymphatic system cancer, fact proven experimentally on rats of the Sprague-Dawley species, have inhibiting action upon the thyroid gland and turning pro-vitamins A into vitamins A, reducing the vitamin content belonging to A group, generating also the absorption reduction of fats and proteins.

- Have strong vasodilating action, which in the case of acute intoxication determines collapse.
In the case of toxic doses, nitrite action appears at the digestive apparatus and kidneys level, causing colic, diarrhea, poly-urea, collapse. Because of the nitrite getting into blood in toxic doses and their combining with hemoglobin anoxia is produced which is expressed by anxiety states, polypnoea, dyspnoea, cyanotic mucous membranes. It is noticed accelerated heart, venous pulse, trembling, astasia, muscular cramps, and diarrhea. After a commotion stage death occurs, the evolution being often shortened by the peripheral collapse, consecutive to the vasodilating effect of nitrites.
The nitrite quantity tolerated daily by the organism is 0.4-0.8 mg/kg-body. The lethal doses for grown-up may vary between 180 mg and 2500 mg. The persons with reduced gastric secretion and active micro-flora in the superior part of the gastrointestinal tract (aerial part) may get intoxicated easier with nitrites.

- In combination with amines, they produce nitrosamines with toxic, teratogen, mutagen and cancer generating action at different species of animals, birds, fish, human. The nitrosamines problem and its solution come across two difficulties that are not encountered at the same measure to the other noxious residua. Nitrosamines may appear on one hand not only in food produces (exogenous origin), but in the digestive apparatus too, especially in the stomach (endogenous origin).

Paradoxically the nitrate quantities taken by the consumer from plants and water are much more over the sum of all other quantities of nitrates and nitrites derived from food conditioned with these additives.

Experimental

The nitrite concentration in fresh produces of vegetable origin is reduced, deriving generally from nitrates, the latter ones being reduced by endogenous reductases.

Salad (Lactuca Sativa), spinach (Spinacia oleracea) cultivated on manure fallow were analyzed.

The paper focused on the research regarding the nitrite content modification in the following conditions:

- content of nitrites in fresh salad, spinach;
- content of nitrites in salad, spinach after three-day refrigeration;
- content of nitrites in salad, spinach after 7-day refrigeration.

The spectrophotometric Greiss reagent method was used to determine the content of nitrites. Series of 20 determinations were made on produces of the same lot. The standard curve was obtained by determining extinctions of a standard solution series containing 1;2;3;4;5;6;7 ml $NaNO_2$ solution (1ml of working solution contains 0,003 mg $NaNO_2$) which is completed to volume of 10 ml with bi-

distilled water , To which 10 ml of Griess reagent is added. The coloration intensity is read after 20 minutes at wavelength of 530nm.

Figure 1 shows the results obtained for the sample of fresh salad.

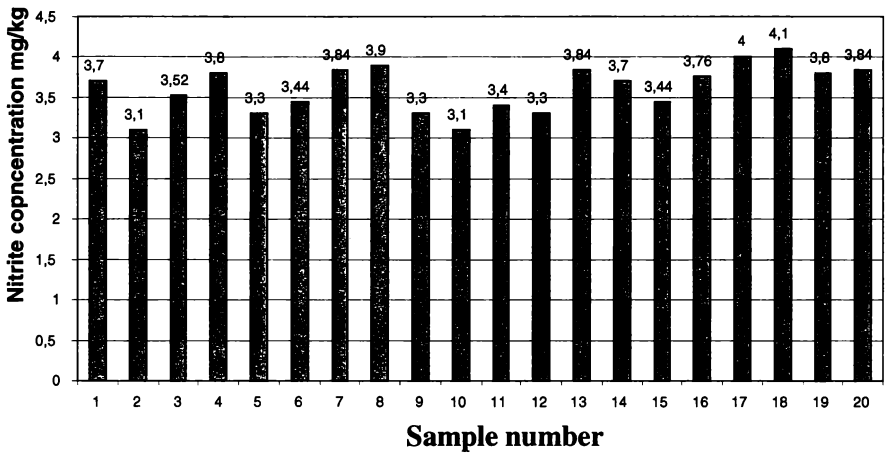


Fig. 1: Nitrite Content Variation in Fresh Salad

The nitrite content in the fresh salad lot varies ion the range 3, 1-4, 1 mg/kg. The average value of nitrite content is of 3, 6 mg/kg produce. The values resulted from determinations are in the admitted limit of nitrite content.

Figure 2 shows the results obtained from determinations of nitrite content for the three-day refrigerated salad.

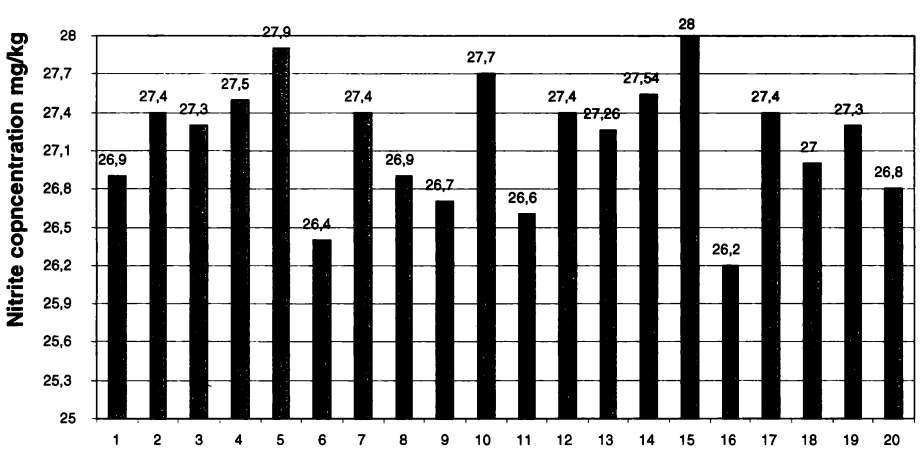


Fig. 2: Nitrite content variation in the three-day refrigerated salad

The nitrite content in the lot of three-day refrigerated salad varies in the range 4, 8-6,1 mg/kg. The average value of determinations is of 5,3 mg/kg. A nitrite content increase of about 2 mg/kg as compared to the fresh produce is noticed, due to the activity of endogenous reductases.

The nitrite content in the lot of seven-day refrigerated salad varies between the range 26, 4-27,9 mg/kg. The average value of determinations is of 27, 21 mg/kg produce.

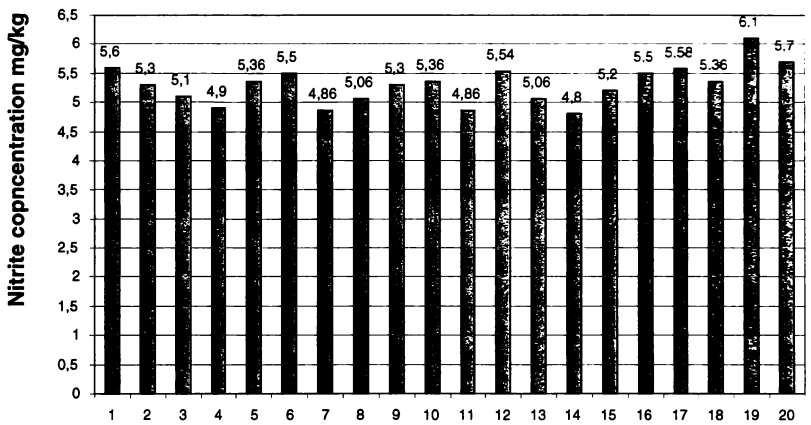


Fig. 3: Nitrite content variation in seven-day refrigerated salad

A fast increase of nitrite content was noticed, from 3,6 mg/kg in fresh produce to 27,21 mg/kg after 7-day refrigeration, an increase of approximately 8 times as compared to the initial value, figure3.

The same determinations were made on spinach too, this one being one of the most consumed vegetables, in fresh, refrigerated or frozen state.

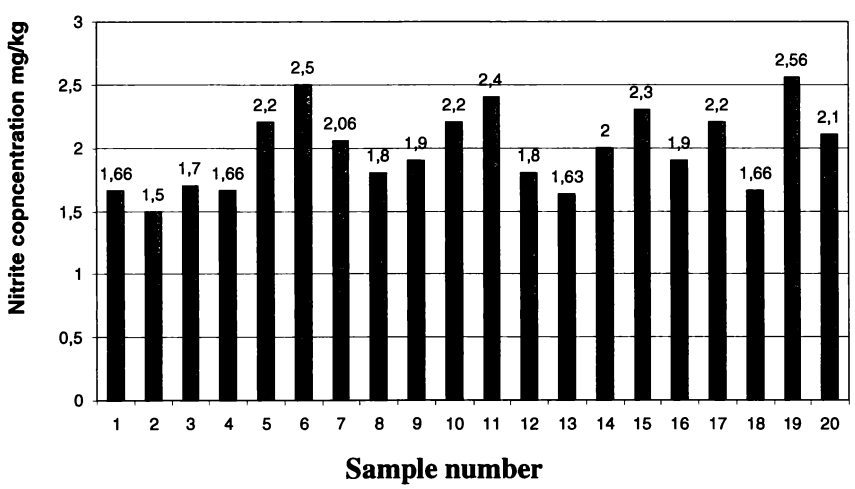


Fig. 4: Nitrite content variation in fresh spinach

The nitrite content in the fresh spinach lot varies in the range 1, 5- 2,56 mg/kg. The average value of nitrite content is of 1, 99 mg/kg. The values are in the admitted limit of nitrite content.

The nitrite content in the three-day refrigerated spinach varies in the range 2,6- 3,48 mg/kg. The average value of determination is 2, 87 mg/kg, value which is in the admitted limit.

A nitrite content increase of 0, 88 mg/kg is noticed as compared to the fresh produce. The nitrite content in the 7-day refrigerated spinach varies in the range 5, 54- 6, 9 mg/kg. The average

value of determinations is of 6, 20 mg/kg. A nitrite content increase of 4, 21 mg/kg is noticed as compared to the fresh produce.

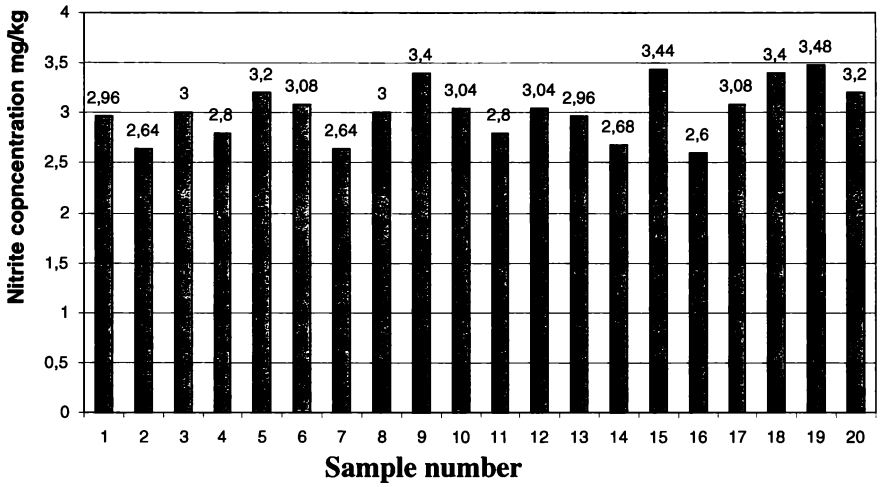


Fig. 5: Nitrite content variation in the three-day refrigerated spinach

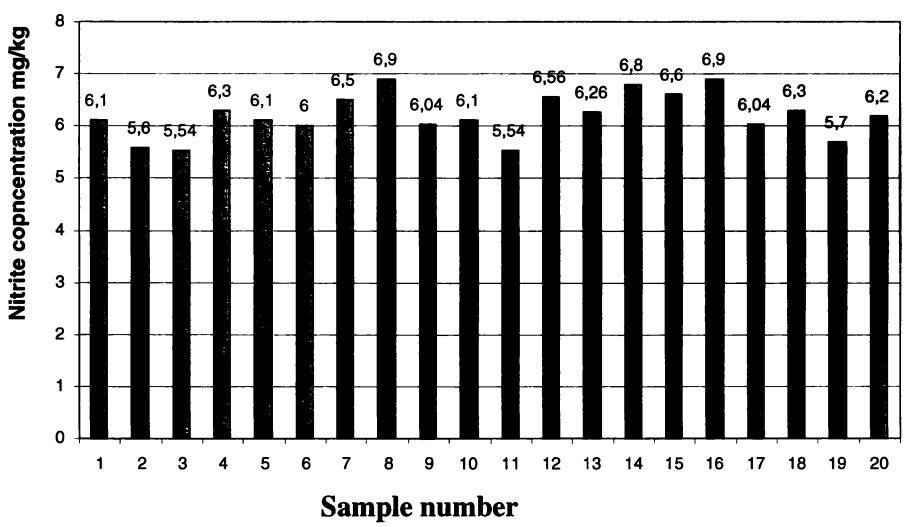


Fig. 6: Nitrite content variation in the 7-day refrigerated spinach

Conclusions:

- Vegetables may be an important source of nitrites, their quantity increases during refrigeration due to the nitrate turning into nitrite under the reducing bacteria influence.
- When salad and spinach are stored for 3-7 days, a nitrite content increase of 5-8 times higher than the content in fresh state is noticed.
- Consequently, it is recommended that these vegetables should be consumed in fresh state only in order to avoid the negative effects upon health, the stronger the effects are, the lower age of the consumer is.

Bibliography:

1. SR 3048-2:1996. Apa potabila. Determinarea azotitilor.
2. STAS 89000/2-71. Ape de suprafata si ape uzate. Determinarea azotitilor
3. Banu, C. Aditivi si ingrediente pentru industria alimentara, Editura Tehnica, Bucuresti, 2000
4. Hansen M, E for additives, Ed. Hammersmith, London, 1998