

PRESENCE OF NITRATE AND NITRITE IN VEGETABLES GROWN FOR SELF-CONSUMPTION

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Abstract

The presence of nitrate in vegetables has been described in a large number of scientific papers. Our paper focused on the presence of nitrate and nitrite anions in vegetables grown for self-consumption. Measurement of nitrite and nitrate content was made using an HPLC-UV/VIS method (UV detection for nitrate and VIS detection for nitrite after derivatization with sulphanilic acid and 1-naphthylamine). Very low concentrations of nitrate were found in most of the samples we tested, with very few exceptions. The lowest concentrations found in nitrate rich vegetables were: < 0.6 mg/kg, 5.50 mg/kg, 2.17 mg/kg, < 0.6 mg/kg and 202.14 mg/kg for lettuce leaves (*Lactuca sativa*), beetroot root (*Beta vulgaris*), carrot root (*Daucus carota* subsp. *sativus*), parsley root (*Petroselinum crispum*) and radish root (*Raphanus sativus*), respectively. Even lettuce proved to contain in many cases unquantifiable concentrations of nitrate (LOQ = 0.6 mg/kg). The results show that EU regulations regarding the nitrate concentrations of vegetables are very permissive. Nitrite was quantifiable in most samples (LOQ = 6 µg/kg). No correlation was observed between nitrate and nitrite content of the vegetables.

Rezumat

Prezența ionului nitrat în produse vegetale a fost descrisă într-un număr considerabil de lucrări științifice. În lucrarea de față am dorit determinarea ionului nitrat și a ionului nitrit în legume cultivate pentru consum propriu. Determinarea concentrațiilor ionilor nitrat și nitrit s-a făcut folosind o metodă HPLC-UV/VIS (dectecție în UV pentru ionul nitrat și dectecție VIS pentru ionul nitrit derivatizat cu acid sulfanilic și 1-naftilamină). Concentrații reduse de nitrat au fost găsite în majoritatea probelor testate. Cele mai mici concentrații de nitrat găsite în legume considerate bogate în nitrat sunt: < 0,6 mg/kg, 5,50 mg/kg, 2,17 mg/kg, < 0,6 mg/kg and 202,14 mg/kg pentru frunze de salată (*Lactuca sativa*), rădăcini de sfeclă roșie (*Beta vulgaris*), rădăcini de morcov (*Daucus carota* subsp. *sativus*), rădăcini de pătrunjel (*Petroselinum crispum*) și ridichi (*Raphanus sativus*). Salata a conținut în multe cazuri cantități necuantificabile de nitrat. Rezultatele arată că normele UE referitoare la prezența ionului nitrat în legume sunt permissive. Ionul nitrit a fost cuantificabil în majoritatea cazurilor (LOQ = 6 µg/kg). Nu s-au înregistrat corelații între concentrația nitritului și a nitrului în plantele testate.

Keywords: nitrate, nitrite, vegetables, lettuce

Introduction

Earlier surveys indicated that high values of nitrate anion (in order of magnitude of grams/kg) can be found in dill leaves (*Anethum graveolens*), lettuce leaves (*Lactuca sativa*), cabbage leaves (*Brassica oleracea* var. *capitata*), spinach leaves (*Spinacia oleracea*), and carrot root (*Daucus carota* subsp. *sativus*) and beetroot root (*Beta vulgaris*) [19, 22, 23, 25]. Surveys assessing the average daily intake of nitrate and nitrite identified as main source of exposure the vegetables, especially the lettuce [23, 24]. In the case of nitrite measurements, most of the samples, if not all, fall under the detection or quantification limits [12, 16, 22, 23].

Nitrate itself is generally considered harmless in low concentrations but is converted into nitrite by

the bacteria found in the oral cavity. Nitrite can induce methemoglobinemia which can result in tissue hypoxia and possible death [6, 18, 21], and can interact with secondary or N-alkyl-amides to form N-nitroso carcinogens [9, 13, 28]. Other new toxicological problems associated with high nitrate and nitrite intake are: neural tube defects [4, 11] and thyroid cancer [27].

Even if there is evidence proving that nitrite and nitrate are a health hazard, recent articles point out possible positive effects of nitrite and nitrate on human health related to the increased intake of these anions [1, 10, 14, 20]. Even when considering beneficial health effects, exceeding of the acceptable daily intake (ADI) was not recommended. If we consider the lettuce that contains about 8 g/kg

nitrate (the highest concentration ever measured), one can observe that by consuming only 33 g of this vegetable, a 70 kg person can exceed the ADI set for this substance [5]. The European Commission regulates nitrate content in lettuce (2 - 4.5 g kg⁻¹ depending on the culture type and harvesting period) and spinach (2 - 3 g kg⁻¹) [19]. It's interesting to note that the limits of nitrate in drinking water are far more restrictive than those stated for vegetables, even if the bioavailability from vegetables and water is not significantly different [26].

Because most of the available studies on nitrate content of vegetables were carried out on mass produced vegetables we decided to perform similar measurements using home-grown vegetables. The nitrite content of the vegetables was also measured with a recently developed suitable method [3].

Materials and Methods

The analyses of samples were performed on a Merck Hitachi L-7000 HPLC system with a diode array detector (DAD) detector using a validated HPLC-UV/VIS method [3]. The mobile phase consisted of a solution of tetrabutylammonium hydroxide 5 mM, brought to pH 2.5 with sulphuric acid, in which methanol and acetonitrile were added as organic modifiers in a gradient mode. Detection was made at 222 nm for nitrate and 520 nm for nitrite, after a derivatization using the Griess reaction. Reverse phase C18 was used as a

stationary phase. Spiked sample technique (4 concentration levels) was used for the measurement of accuracy (97-104% and 98-104% for nitrite and nitrate, respectively) and precision (RSD less than 5%). Limits of quantification were 0.6 mg/kg and 6 µg/kg for nitrate and nitrite anions, respectively. Both compounds were detected simultaneously in a single run [3].

Vegetable samples were obtained from farmers that produce vegetables for self-consumption in rural areas of Mureş and Harghita counties, Romania. Two types of vegetal samples were analysed (when available): fresh vegetables (sample treated within 2 hours after harvesting) and preserved vegetables (samples stored in basements at least 3-4 months after harvesting). Soil or other residues were removed from the vegetables before sample treatment by rinsing them with purified water. The samples were ground and the resulted juice was kept frozen (-20°C) until analysis. Vegetable juices were diluted, proteins were precipitated with acetonitrile, and after centrifugation were subjected to a derivatization process using sulphanilic acid and 1-naphthylamine [3].

Results and Discussion

The nitrite and nitrate content of vegetables is shown in Table I. The distribution of nitrate content in the analysed vegetables is shown in Figure 1.

Table I
Nitrite and nitrate concentrations measured in vegetable samples

Sample	N ⁴	Nitrite (µg/kg)			Nitrate (mg/kg)		
		Average	Min	Max	Average	Min	Max
white potato tuber (<i>Solanum tuberosum</i>) (preserved)	6	99.6	32.8	170.1	74.78	1.75	354.15
white potato tuber (<i>Solanum tuberosum</i>) (fresh)	20	60.8	7.8	167.7	12.67	1.61	42.89
red potato tuber (<i>Solanum tuberosum</i>) (preserved)	8	85.4	7.2	162.9	-	BLQ	78.57
red onion bulb (preserved)	5	- ³	BLD ¹	BLQ ²	1.13	0.64	1.80
white onion bulb (<i>Allium cepa</i>) (preserved)	6	-	BLD	BLQ	-	BLQ	0.92
garlic clove (<i>Allium sativum</i>) (preserved)	5	-	BLQ	BLQ	128.80	58.58	199.03
beetroot root (<i>Beta vulgaris</i>) (preserved)	5	171.5	15.6	212.0	1130.83	5.50	1647.4
beetroot root (<i>Beta vulgaris</i>) (fresh)	20	24.8	8.9	79.3	396.28	39.44	600.92
carrot root (<i>Daucus carota subsp. sativus</i>) (preserved)	10	47.84	16.6	72.5	62.28	2.17	280.97
parsley root (<i>Petroselinum crispum</i>) (preserved)	5	27.0	15.9	31.2	-	BLQ	173.28
parsley root (<i>Petroselinum crispum</i>) (fresh)	20	-	BLQ	349.6	82.28	12.15	151.89
radish root (<i>Raphanus sativus</i>)	22	33.4	11.0	193.3	413.39	202.14	717.19
tomato (<i>Solanum lycopersicum</i>)	7	-	BLQ	15.2	-	BLQ	4.79
cucumber (<i>Cucumis sativus</i>)	5	18.9	16.3	21.1	37.63	27.06	52.75
lettuce (<i>Lactuca sativa</i>)	31	-	BLQ	369.2	-	BLQ	3140.0
apple (<i>Malus domestica</i>) (fresh)	6	18.1	12.9	20.6	-	BLQ	0.41

¹BLD – below limit of detection

²BLQ – below limit of quantification

³ – where at least one sample had BLQ concentration, no average concentration was computed

⁴ – number of samples tested

Most of the vegetables contained measurable amounts of nitrite. Interestingly, white onion bulb (*Allium cepa*), red onion bulb (*Allium cepa*) and garlic clove (*Allium sativum*) (16 samples in total)

contained no measurable amounts of nitrite at a level of quantification of only 6 µg/kg. From a toxicological point of view, the nitrite content was completely insignificant in all samples. No

correlation was found between the nitrite content of the samples and the corresponding nitrate content. Many studies found nitrite below the limit of detection in most, if not all, of the samples. Sometimes, with incidences over 15%, relatively high nitrite contents were measured in some vegetable and fruit samples. In our study such high concentrations were never found. In addition, worth to mention that the method we used is far more sensitive (LOQ of 6 $\mu\text{g/kg}$ versus thousands of $\mu\text{g/kg}$) and far more specific (retention time + colour reaction versus only retention time) than those used before (HPLC-UV or HPLC-VIS with post-column derivatization) [7, 8, 12, 15-17, 22]. In cases where high nitrite content is reported, a bacterial contamination should be considered instead of a natural nitrite occurrence.

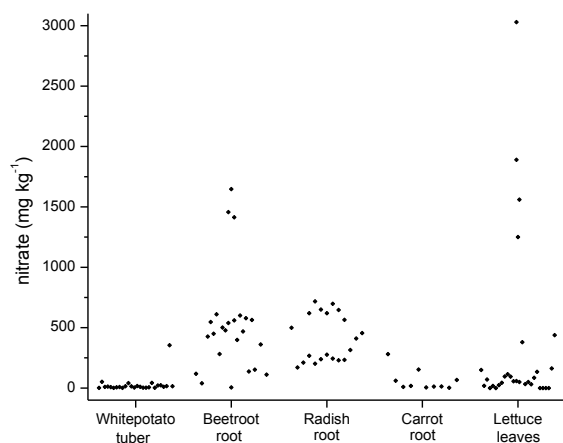


Figure 1.

Distribution of the nitrate concentration in the tested vegetables

As expected, lettuce leaves, beetroot root, radish root and white potato tuber had the highest concentrations of nitrate. However, concentrations measured in our study are lower than those recorded during studies made on mass produced vegetables. The nitrate content of vegetables had extremely large variations for most of the tested sample types. The largest variations were recorded in the cases of preserved white potato tuber (1.75 - 354.15), beetroot root (5.50 - 1647.40), carrot root (2.17 - 280.97) and lettuce (BLQ - 3140.0). It is important to note that, in this study, many vegetables considered as high nitrate source (especially lettuce and beetroot) contained unexpectedly small amounts of nitrate. Only 2/31 samples of lettuce contained nitrate amounts higher than 3000 mg/kg, 5/31 contained more than 1000 mg/kg and 18/31 contained less than 100 mg/kg. The impossibility to quantify or detect the amount of nitrate, at a quantification limit of 0.60 mg/kg, was encountered in 6/31 samples of lettuce. The low concentration of nitrate, found in the samples

we analysed, can be explained by the fact that all samples were harvested from small vegetable gardens where vegetables are grown for self-consumption and not for commercial purposes. These vegetables were grown by traditional farmers who do not accept the idea of using fertilizers or other chemicals. The fact that the vegetables were fresh or were preserved in good conditions might explain why nitrite was present only in very low amounts in our study.

These findings suggest that plants as lettuce, beetroot, and carrot should not be considered as "endemic" carriers of nitrate, and that good agricultural practice could significantly reduce the amount of nitrate in vegetables. As far as we know, such low concentrations of nitrate in beetroot and lettuce were not found by previous researchers. Unfortunately, we did not find any rational explanation why such large variation occurred in the content of nitrate in vegetables grown for self-consumption, because no observable differences were found between the methods of growers regarding the treatment they subject their vegetables to. Further studies are needed to identify these minimal differences in the conditions that influence the content of nitrate in the vegetables.

Conclusions

Our study proved that it is possible to grow safe vegetables with minimum nitrate concentrations, which are completely insignificant from a toxicological point of view. The high tolerance of authorities regarding the presence of nitrate in plant material could not be explained by an endemic presence of nitrate in some types of vegetables. Therefore, more restrictive nitrate regulations, as those imposed for tap water, should be also enforced for vegetables.

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