

## NITRATES AND NITRITES IN SELECTED VEGETABLES PURCHASED AT SUPERMARKETS IN SIEDLCE, POLAND

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### ABSTRACT

**Background.** Vegetables constitute a vital part of the human diet, being the main source of minerals, vitamins, dietary fibre and phytochemicals. They however, also contain nitrates and nitrites, which adversely affect human health.

**Objective.** To determine nitrate and nitrite content in selected vegetables purchased at supermarket chains in Siedlce and to assess their impact on consumer health.

**Material and methods.** Vegetable samples were purchased from local supermarkets in Siedlce, town situated in the Mazovian province (Voivodeship) of Poland. These consisted of 116 samples of nine vegetables types including butterhead and iceberg lettuce, beetroot, white cabbage, carrot, cucumber, radish, tomato and potato collected between April and September 2011. Concentrations of nitrate and nitrite were determined by standard colorimetric methods used in Poland, with results expressed as mg per kg fresh weight of vegetables.

**Results.** Nitrate concentrations varied between 10 mg·kg<sup>-1</sup> to 4800 mg·kg<sup>-1</sup>. The highest mean nitrate concentrations were found in radishes (2132 mg·kg<sup>-1</sup>), butterhead lettuce (1725 mg·kg<sup>-1</sup>), beetroots (1306 mg·kg<sup>-1</sup>) and iceberg lettuce (890 mg·kg<sup>-1</sup>), whereas the lowest were found in cucumber (32 mg·kg<sup>-1</sup>) and tomato (35 mg·kg<sup>-1</sup>). Nitrite levels were also variable; the highest concentrations measured were in beetroot (mean 9.19 mg·kg<sup>-1</sup>) whilst much smaller amounts were present in carrot, cucumbers, iceberg lettuce, white cabbage, tomatoes and potatoes. The daily adult consumption of 100 g amounts of the studied vegetables were found not exceed the ADI for both nitrates and nitrites.

**Conclusions.** Findings indicated the need for monitoring nitrate and nitrite content in radishes, butterhead lettuce and beetroot due to consumer health concerns.

**Keywords:** nitrate, nitrite, vegetables, ADI, acceptable daily intake

### STRESZCZENIE

**Wprowadzenie.** Warzywa zajmują ważne miejsce w diecie człowieka, ponieważ są głównym źródłem minerałów, witamin, błonnika pokarmowego i substancji fitochemicznych. Warzywa są także źródłem azotanów(V) i azotanów(III), które mogą wpływać negatywnie na zdrowie człowieka.

**Cel.** Celem badań było określenie zawartości azotanów(V) i azotanów(III) w wybranych warzywach zakupionych w sieci handlowej na terenie Siedlec.

**Material i metody.** Warzywa zakupiono w lokalnych supermarketach w Siedlcach (woj. Mazowieckie). Przebadano 116 próbek dziewięciu gatunków warzyw takich jak: sałata masłowa i lodowa, burak ćwikłowy, kapusta biała głowiasta, marchew, ogórek, rzodkiewka, pomidor i ziemniak w okresie od kwietnia do września 2011 roku. Azotany(V) i azotany(III) w warzywach oznaczono metodą kolorymetryczną zgodnie z Polską Normą. Wyniki podano w mg·kg<sup>-1</sup> świeżej masy warzyw.

**Wyniki.** Zawartość azotanów(V) w badanych warzywach wahała się od 10 mg·kg<sup>-1</sup> do 4800 mg·kg<sup>-1</sup>. Największą średnią zawartość azotanów(V) stwierdzono w rzodkiewce (2132 mg·kg<sup>-1</sup>) i w sałacie masłowej (1725 mg·kg<sup>-1</sup>). Znaczne ilości azotanów odnotowano także w buraku ćwikłowym (1306 mg·kg<sup>-1</sup>) i w sałacie lodowej (890 mg·kg<sup>-1</sup>). Najmniejszą średnią zawartość azotanów(V) stwierdzono w ogórkach (32 mg·kg<sup>-1</sup>) oraz w pomidorach (35 mg·kg<sup>-1</sup>). Zawartość azotanów (III) w badanych warzywach była różnicowana. Największą zawartość azotanów(III) odnotowano w buraku ćwikłowym (średnio 9.19 mg·kg<sup>-1</sup>), a znacznie mniejsze w marchwi, ogórkach, sałacie lodowej, kapuście głowiastej białej, pomidorach i ziemniakach. Spożycie po 100 g dziennie badanych warzyw przez osobę dorosłą dostarcza azotany(V) i azotany(III) w ilości nieprzekraczającej wartość ADI.

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**Wnioski.** Otrzymane wyniki wskazują na potrzebę monitoringu zawartości azotanów(V) i azotanów(III) w rzodkiewce, sałacie masłowej i buraku ćwikłowym w trosce o ochronę zdrowia konsumenta.

**Słowa kluczowe:** azotany(V), azotany(III), warzywa, akceptowane dzienne pobranie, ADI

## INTRODUCTION

Vegetables are undoubtedly vital for human nutrition. A vegetable rich diet provides many health-promoting substances such as minerals, vitamins (A, C, B<sub>1</sub>, B<sub>6</sub>, B<sub>9</sub>, E), dietary fibre and phytochemicals. A daily diet that includes vegetables is strongly associated with an overall good health and wellbeing, improvement of gastrointestinal function and vision, reduced risks of heart disease, stroke, diabetes, anaemia, rheumatoid arthritis, peptic ulcer and some forms of cancer [3]. Despite the nutritional benefit of eating vegetables, they also contain substances that adversely affect human health such as nitrates and nitrites. In themselves, the former are relatively non-toxic but about 5–7% of all ingested nitrate is reduced by nitrate-reducing microflora to the more toxic nitrites in the saliva and gastrointestinal tract. In persons with a high rate of conversion this value can reach 20% [17]. Nitrites can adversely affect human health where the best known effect of nitrites is methaemoglobinaemia in infants [4, 11, 17]. Nitrites can also react with amines or amides to form carcinogenic, mutagenic and teratogenic N-nitroso compounds [7, 20, 26]. They may cause irreversible changes in the brain, disturb thyroid functioning impair the absorption of proteins and fats. Nitrites may affect carotenoid, vitamin A and the B group vitamins degradation, thus decreasing the nutritional value of consumed vegetables [21]. Nevertheless, recent studies suggest that a diet containing nitrate and nitrite rich vegetables is beneficial for health due to their conversion to nitric oxide which prevents cardiovascular disease and microbial infection, reduces hypertension, affords gastric protection and serves as a nutritional aid for maintaining optimal cardiovascular health [11, 17, 20].

Vegetables form the major daily intake source of nitrates in humans supplying about 70-90 % of the total intake [11, 26, 28]. This intake depends on the type of vegetables consumed, nitrate vegetable levels and the amount of vegetables actually consumed [11]. Nitrate and nitrite accumulation is found not just to depend on vegetable type but also on many pre- and post-harvest factors [20, 27]. When large amounts of vegetables are consumed, which have accumulated high amounts of nitrates, a need thus arises for the systematic control of the dietary intake of nitrogenous compounds [10].

The aim of the study was therefore to determine nitrate and nitrite content in selected vegetables purchased in supermarket chains in a typical large town (Siedlce) representing Poland.

## MATERIAL AND METHODS

Vegetables in 0.5 to 1-2 kg amounts were bought at local supermarkets in Siedlce, between April and September 2011, consisting of 116 samples from nine different types of vegetable species that included butterhead and iceberg lettuce (*Lactuca sativa* L. var. *capitata*), white cabbage (*Brassica oleracea* L. var. *capitata* L. f. *alba*), carrot (*Daucus carota* L.), cucumber (*Cucumis sativus* L.), radish (*Raphanus sativus* L.), beetroot (*Beta vulgaris* L. var. *conditiva* Alef.), tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.). All were in a suitable state for human consumption i.e. fresh and undecayed. Nitrate and nitrite levels were determined according to a standard colourimetric method used in Poland [18] where vegetables were boiled in hot water until sufficiently cooked, followed by a protein precipitation step and filtration. The *Griess* reagent was used for measuring nitrites and in the case of nitrate levels, a previous reduction of nitrate to nitrite was performed. All analyses were in duplicate and results expressed in mg per kg of vegetable fresh weight.

## RESULTS AND DISCUSSION

Nitrate concentrations were found to vary between 10 mg·kg<sup>-1</sup> to 4800 mg·kg<sup>-1</sup> (Table 1). The highest mean concentration of nitrates were in radishes (2132 mg·kg<sup>-1</sup>) and in butterhead lettuce (1725 mg·kg<sup>-1</sup>), with substantial amounts of nitrates noted in beetroots (1306 mg·kg<sup>-1</sup>) and iceberg lettuce (890 mg·kg<sup>-1</sup>). The lowest mean concentration of nitrates were found in cucumbers (32 mg·kg<sup>-1</sup>) and tomatoes (35 mg·kg<sup>-1</sup>). These results were found to be in accordance with how vegetables are divided by their ability to accumulate nitrates [20] (Table 2). Highest levels of nitrate were observed in vegetables of the families; *Brassicaceae* (radish, cabbage), *Chenopodiaceae* (beetroot) and *Asteraceae* (lettuce). The smallest amounts of nitrates were accumulated by vegetables of the family *Solanaceae* (potato, tomato) and *Curcubitaceae* (cucumber). It is generally seen that vegetables with edible roots, stems and/or leaves accumulate the most nitrate whereas those of a consumable fruit form accumulate least [31]. Nitrate concentrations have been shown to differ amongst the edible parts of plants [13, 19, 28, 31], and indeed vegetable organs can be listed by their decreasing nitrate content in the following order: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed [19].

Table 1. Nitrate concentrations in vegetables

Vegetables	Number of samples	NO <sub>3</sub> <sup>-</sup> (mg·kg <sup>-1</sup> fresh weight)		
		Range	Mean	SD*
Beetroot	20	480–2000	1306	405
White cabbage	12	116–1260	436	356
Carrots	13	11–259	82.2	84.8
Cucumber	6	24–40	32	5
Butterhead lettuce	12	530–4440	1725	1101
Iceberg lettuce	15	125–1580	890	541
Radishes	16	710–4800	2132	1427
Tomato	13	10–100	35	32
Potato	9	11–90	54	32

\*SD–Standard Deviation

Nitrate levels in beetroots ranged from 480 and 2000 mg·kg<sup>-1</sup> (Table 1) and in 15 out of the 20 studied samples these exceeded 1000 mg·kg<sup>-1</sup> whilst in 8 (i.e. 40%) they exceeded 1500 mg·kg<sup>-1</sup>. Similar concentrations of nitrates has been found in commercially available beetroots from the Polish towns of Legionowo, Serock and Olsztyn [10, 16]. In white cabbage, nitrate levels were between 116 to 1260 mg·kg<sup>-1</sup> with a mean of 435 mg·kg<sup>-1</sup> (Table 1). Only one out of twelve cabbage samples had a nitrate concentration more than 1000 mg·kg<sup>-1</sup>. These results are similar to the *Murawa et al* [16] study and smaller concentrations were found by *Gajewska et al* [9]. Studies abroad demonstrated similar nitrate concentrations in cabbages from Portugal (472 and 547 mg·kg<sup>-1</sup>), France (498 mg·kg<sup>-1</sup>), Turkey (510 mg·kg<sup>-1</sup>) and Estonia (437 mg·kg<sup>-1</sup>) [1, 14, 15, 22].

In butterhead lettuce, nitrates levels varied between 530 to 4400 mg·kg<sup>-1</sup> (Table 1). One sample obtained in the summer (June) exceeded the 3000 mg·kg<sup>-1</sup> nitrate standard value for lettuces grown in the open air between April 1st to September 30th [6]. Eight out of twelve lettuce samples had nitrate concentrations over 1000 mg·kg<sup>-1</sup> with two that were above 2000 mg·kg<sup>-1</sup>. Such high concentrations of nitrates in lettuce were also found by other authors [2, 8, 9, 14–16]. A monitoring study by *Gajda-Wyrębek et al* [8] in Poland showed that the permissible nitrate content was exceeded in 5.6% samples of lettuce grown under cover and in 9.9% samples of lettuce grown in the open air. A French study found that the maximum nitrate content in lettuce was 5600 mg·kg<sup>-1</sup> with a mean of 1974 mg·kg<sup>-1</sup> [14] whilst in southern Turkey the maximum was 3809 mg·kg<sup>-1</sup> with a mean of 1439 mg·kg<sup>-1</sup> [15].

EC regulations have set nitrate levels in iceberg lettuce ranging from 125 to 1580 mg·kg<sup>-1</sup> and should never exceed values of 2500 mg·kg<sup>-1</sup> for iceberg lettuce grown under cover and 2000 mg·kg<sup>-1</sup> for that grown in the open air [6]. Four out of the fifteen analysed samples (26.7%) of iceberg lettuce showed the concentration of nitrates above 1000 mg·kg<sup>-1</sup>. The monitoring performed in Poland during 2007–2008 by *Gajda-Wyrębek et al*

[8] demonstrated that the allowable nitrate content in iceberg lettuce was exceeded in only 3.9% of the samples studied.

Nitrate content in radishes was found in the 710 to 4800 mg·kg<sup>-1</sup> range (Table 1). Levels above 1000 mg·kg<sup>-1</sup> were observed in 13 out of 16 analysed samples; five of which had values 3.5 to 5 times higher. Such results are much higher than those obtained in other studies by *Czech and Rusinek* (542.8–2890.3 mg·kg<sup>-1</sup>) and *Tietze et al* (1548.9 mg·kg<sup>-1</sup>) [2, 23]. Another study by *Wolejko et al* [29], demonstrated that the highest nitrate content in radishes grown in allotments was 4400 mg·kg<sup>-1</sup>. Furthermore, *Menard et al* [14] found that the maximum nitrate content of nitrates in radish consumed in France was 5000 mg·kg<sup>-1</sup> with a mean content of 1860.7 mg·kg<sup>-1</sup> whilst *Mor et al* [15] found 9259 mg·kg<sup>-1</sup> of nitrate in radishes grown in southern Turkey.

Table 2. Vegetables classified according to nitrate concentration [20]

NO <sub>3</sub> <sup>-</sup> concentration (mg·kg <sup>-1</sup> fresh weight)	Vegetables
Very low (<200)	artichoke, asparagus, broad bean, brussel sprouts, aubergines, garlic, onion, green bean, melon, mushroom, peas, peppers, potato, summer squash, sweet potato, tomato, watermelon
Low (200–500)	broccoli, carrot, cauliflower, cucumber, pumpkin, chicory
Middle (500–1000)	cabbage, dill, savoy cabbage, turnip
High (1000–2500)	celeriac (celery root), chinese cabbage, endivie, escarole, fennel, kohlrabi, leaf chicory, leeks, parsley
Very high (>2500)	celery, chervil, water cress, lamb's lettuce, ordinary lettuce, radishes, red beetroot, rocket lettuce, spinach, swiss chard

Much smaller amounts of nitrates were however found in carrots (11–260 mg·kg<sup>-1</sup>), cucumbers (24–40 mg·kg<sup>-1</sup>), tomatoes (10–100 mg·kg<sup>-1</sup>) and potatoes (10–98 mg·kg<sup>-1</sup>) as shown in Table 1, in accordance with the results obtained by *Dymowska-Malesa et al* [5], *Gajewska et al* [9], *Murawa et al* [16] and *Wójcik-Stopczyńska et al* [30].

According to literature data [1], the nitrate content in vegetables can vary between 1 to 10,000 mg kg<sup>-1</sup>, with the highest value of 10.800 mg kg<sup>-1</sup> being noted in samples of celery grown under hydroponic conditions.

The accumulation of nitrates in vegetables depends on many factors; genetic, agricultural (e.g. type of soil, the dose and chemical forms of nitrogen, availability of other nutrients, herbicide application etc.) and those environmental such as air humidity, soil water content and photoperiodicity. Further differences in nitrate accumulation may arise from the time of harvest, vegetation season and storage time [20, 22, 24]. According

to *Wojciechowska* [27], the main factors that contribute towards the accumulation of nitrate in vegetables can be graded as follows: genetic factors –10%, growing period –15%, soil conditions –20%, fertilisation –30% and weather conditions –25%. Of the latter two, nitrogen fertilization and light intensity have been identified in other studies as being the major factors that influence nitrate content in vegetables [20, 27].

The nitrate content in plants also changes with age. Young plants have a higher nitrate content since the reduction of nitrates does not keep pace with the uptake rate of this ion. Moreover, a higher nitrate accumulation is considered typical of plant species with shorter vegetative periods compared to those that take longer to grow [27], as indeed confirmed by the presented study where for example the highest nitrate concentrations were seen in radishes.

Vegetables usually contain small amounts of nitrites but they may also arise from nitrate reduction whenever they are improperly stored. The current study found that vegetable nitrite content was variable. The highest concentrations of nitrites were observed in beetroots (mean  $9.19 \text{ mg}\cdot\text{kg}^{-1}$ ) whilst much smaller amounts were noted in carrots, cucumbers, iceberg lettuce, cabbage and tomatoes (Table 3). Small amounts of nitrites were also detected in potatoes. The presented results are however markedly higher than those of other Polish studies [2, 9, 25]. Very high nitrite concentrations of nitrites have although been demonstrated in radishes ( $19$  to  $60 \text{ mg}\cdot\text{kg}^{-1}$ ) and in lettuce ( $21$  to ca.  $30 \text{ mg}\cdot\text{kg}^{-1}$ ) grown in allotments as published by *Wolejko* et al [29]. Generally, nitrite content in vegetables usually falls within the range of  $1$ – $2 \text{ mg}\cdot\text{kg}^{-1}$  but it may exceed  $20 \text{ mg}\cdot\text{kg}^{-1}$  and reach up to  $60 \text{ mg}\cdot\text{kg}^{-1}$  in potatoes and  $57 \text{ mg}\cdot\text{kg}^{-1}$  for turnip sprouts [2, 20]. In France the maximum content of nitrites in lettuce and spinach was  $25 \text{ mg}\cdot\text{kg}^{-1}$  and  $220 \text{ mg}\cdot\text{kg}^{-1}$  respectively [14]. Such high concentrations of nitrites may result from nitrate reduction occurring during improper storage, particularly if this is for long periods at temperatures above those recommended or without access to oxygen [27].

Table 3. Nitrite concentrations in vegetables

Vegetables	Number of samples	$\text{NO}_2^- \text{ mg}\cdot\text{kg}^{-1}$ fresh weight		
		Range	Mean	SD
Beetroot	20	2.50–15.00	9.19	3.65
White cabbage	12	0.60–1.20	0.88	0.23
Carrots	13	<0.40–0.80	0.46	0.15
Cucumber	6	<0.40–0.80	0.50	0.21
Butterhead lettuce	12	<0.40–3.33	1.64	0.95
Iceberg lettuce	15	<0.40–1.22	0.60	0.31
Radishes	16	<0.40–3.20	1.90	1.14
Tomato	13	<0.40–1.40	0.82	0.46
Potato	6	<0.40–0.80	0.50	0.18

Various EC and other governmental bodies have developed toxicity values for dietary nitrate and nitrite intake that includes food and water supplies. The Acceptable Daily Intake (ADI) of nitrates has been established as being  $0$ – $3.7 \text{ mg}\cdot\text{kg}^{-1}$  body weight by the European Commission's Scientific Committee on Food (SCF) [17] and the Joint Expert Committee of the Food and Agriculture (JECFA) organisation belonging to the United Nations/World Health Organization (WHO) [7]. The daily human intake of nitrate has been shown to occur from three major sources: vegetables, drinking water and meat products. Thus, assuming a daily consumption of  $0.5 \text{ kg}$  fresh vegetables per person, then the maximum nitrate levels in vegetables should be  $432 \text{ mg}\cdot\text{kg}^{-1}$  [4].

Results so presented in this study demonstrate that the consumption of only  $100 \text{ g}$  of vegetables delivers  $40.1$  to  $96.0\%$  of ADI of nitrates to an adult person weighing  $60 \text{ kg}$ ; vegetables consisting of iceberg lettuce, beetroot, butterhead lettuce or radishes bought between April 1st and September 30th in Siedlce supermarkets.

Both the SCF [17] and JECFA [7] have respectively set an ADI for nitrite at  $0$ – $0.06$  and  $0$ – $0.07 \text{ mg NO}_2^- \cdot \text{kg}^{-1}$  b.w. (body weight). Based on the obtained results it was calculated that an adult consuming only  $100 \text{ g}$  of the studied vegetables can deliver nitrite amounts ranging from trace to  $0.015 \text{ mg NO}_2^- \cdot \text{kg}^{-1}$  b.w.; i.e. up to  $25.0\%$  of the ADI ( $0.06 \text{ mg NO}_2^- \cdot \text{kg}^{-1}$  b.w.). Taking into account that the  $5\%$  ca. of nitrate that are ingested with food is converted to nitrites [17], then an adult consumption of  $100 \text{ g}$  beetroots is equivalent to a daily intake of  $0.124 \text{ mg NO}_2^- \cdot \text{kg}^{-1}$  b.w. which is around twice the estimated ADI. A consumption of  $100 \text{ g}$  of radish delivers  $0.178 \text{ mg NO}_2^- \cdot \text{kg}^{-1}$  b.w. which thus approximately exceeds the ADI value three times. A study by *Wawrzyniak* et al [26] has shown that vegetables and their preserves deliver  $5$ – $8\%$  of the nitrites in a daily student diet.

Measuring just nitrate content in fresh vegetables is however insufficient for estimating amounts ingested as a proportion of the human diet. Nitrate concentrations may be decreased when vegetables are prepared before cooking (e.g. washing and peeling) as well as subsequent thermal processing. The highest nitrate loss after such procedures have been recorded in potato tubers, beetroot and cabbage [10].

As well as being a nitrate source, vegetables can also be the main source of ascorbic acid (vitamin C). The relative levels of these components may be expressed by means of the ascorbate-nitrate index (IAN) [12], where this can be used to evaluate the nutritional quality of vegetables. Thus vegetables can be classified into three groups on the basis of this IAN value according to the health risk;  $<0.5$  indicates a risk,  $>0.5$  and  $<1.0$  is considered harmless whilst  $>1.0$  is absolutely safe. It has also been shown that ascorbic acid may inhibit

nitrosamine synthesis and so lower the risk of methaemoglobinaemia [12].

## CONCLUSIONS

1. Radishes, butterhead and iceberg lettuce together with beetroots accumulated the largest amounts of nitrates whereas tomatoes, cucumbers and potatoes contained the least. The highest nitrite concentrations were found in beetroot, radishes, butterhead lettuce and potatoes.
2. Nitrate content exceeded the permissible standard in only one sample of butterhead lettuce; the rest being acceptable.
3. A daily 100 g consumption of those vegetables studied by an adult does not exceed the ADI values for nitrates and nitrites.
4. The study results demonstrate the need for monitoring nitrate and nitrite contents in radishes, butterhead lettuce and beetroots in order to safeguard consumer health.

## Conflict of interest

The authors declare no conflict of interest.

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