CHUGOKUGAKUEN J. 2004
Vol. 3, pp. 7-10


CHUGOKUGAKUEN
Journal
http://www.cjc.ac.jp/

*Original Article*

# Nitrate in Vegetables

**Yoshihiro Shimada* and Sanae Ko**

*Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, Okayama 701-0197, Japan*

**The nitrate contents of various vegetables were analyzed by ion chromatography. It was shown that tahtsai, spinach, chingentsai and pakchoi contained more than 300 mg of nitrate per 100 g of edible portion. There was a significant difference in the nitrate content of leaf, stem and edible portion of chingentsai between summer and winter, and there was a significant difference in the nitrate content of leaf, stem of tahtsai between summer and winter. There was a significant difference in the nitrate content of chingentsai between hydroponics and soil culture. The procedures of boiling and soaking tended to lower nitrate content.**

**Key Words:** Nitrate, Nitrite, Vegetables, Ion chromatography, hydroponics

## Introduction

Nitrates are comprised of those that occur naturally in various foods and of those that are artificial counterparts found as additives in foods. The presence of nitrates is one of the consequences of the process by which plants absorb the nitrogen element, in the form of $NO_3^-$, from fertilizers or organic materials [1, 2, 3], which are essential to the process of protein synthesis. This process also explains the findings of many studies in which all vegetables were shown to contain nitrates at varying levels ranging from 1 to 10,000 mg/kg.

Until recently, concern over the amounts of nitrate and nitrite in our diet has been due to the relationship between nitrate and nitrite, and infant methemoglobinemia. Currently, the role of nitrites in the formation of the carcinogenic nitrosamines has led to some public apprehension regarding the nitrite content of our food.

Nitrate has a low level of acute toxicity, but it can be transformed into nitrite, which has much higher acute toxicity. It has been estimated that about 4-8% of the nitrate from the diet may be reduced to nitrite by the microflora in the oral cavity [1, 4, 5]. Some studies showed that nitrate exposure is correlated with gastric cancer risk due to the endogenous formation of N-nitroso compounds [6]. The intake of nitrite is normally low compared with toxic level doses, but nitrite in food is considered to be a health problem primarily because its presence both in food and in the human body may lead to the formation of nitrosamines. Experiments in animals have shown that many nitrosamines are potent carcinogens and that they, finally, may also cause cancer in humans. Thus, there is increasing concern about the contamination of foods, especially vegetables [7, 8, 9], with nitrate and nitrite, because vegetables are the largest source of dietary nitrate acounting for more than 90% of nitrate intake [10].

Cooking vegetables tends to lower nitrate content since nitrate is soluble and readily leaches into cooking liquids. Researchers have found that between 14% and

*Corresponding author.
Yoshihiro Shimada, PhD.
Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, 83, Niwase, Okayama 701-0197, Japan
Tel & FAX; +1 86 293 0247

79% of the nitrate contained in fresh vegetables is lost when they are cooked [11, 12].

The objectives of this study were to survey levels of nitrate in ten commercially obtainable fresh vegetables, and to the compare the nitrate contents of two vegetables (chingentsai and tahtsai) in harvest season and the nitrate contents in chingentsai grown by hydroponics and in a soil culture.

Further, the present work describes the effect of cooking in boiling water on the nitrate content of four different vegetables.

## Materials and Method

### *Samples and Chemicals*

Vegetables were purchased from local supermarkets. Fresh vegetables were obtained from 1990 to 1995 and were representative in quality of those available at the time. Fresh vegetables were analyzed on the day of purchase. All chemicals were analytical grade and the water was deionized prior to distillation.

### *Procedure of cooking*

A 250 g sample of each vegetable was placed in 5,000 ml of pure boiling water for the given experimental time (1 or 3 min.). The boiled vegetables were then soaked for the given experimental time (2 or 10 min.). After cooking, the vegetables were wrung by hand to remove excess water.

### *Preparation of sample*

First, samples of tahtsai, spinach, chingentsai and pakchoi were divided into leaf and stem. All vegetables were then chopped into small sections with a kitchen knife. Portions of 5 g each were weighed and were homogenized with 45 ml of water in a mortar. They were then placed in a water bath at 80 °C for 3 min. and centrifuged for 15 min at 3,000 rpm. The supernatant was filtered and diluted 100 times with water and was subjected to ion chromatography.

### *Nitrate analysis*

The ion chromatography system used in this study consisted of a Dionex 2000 i/SP ion chromatograph, a Dionex HPIC-AS4A column (4 × 250 mm) with a IonPac AG4A guard column (4 × 50 mm) and a Chromatocorder 12 integrator. The mobile phase was composed of 1.8 mM sodium carbonate, 1.7 mM sodium hydrogencarbonate at a flow rate of 1.0 ml per min at 30 °C. Nitrate content was calculated from the standard curve prepared with standard sodium nitrate solution.

## Results and Discussion

Results of analyses of 10 fresh vegetables available in local supermarkets from July 1991 to December 1991 are given in Table 1. It was shown that tahtsai, spinach, chingentsai and pakchoi contained more than 300 mg of nitrate per 100 g of edible portion, while Malabar nightshade, Chinese chive, cabbage, lettuce, asparagus bean and garlic contained less than 100 mg of nitrate per 100 g of edible portion. Nitrate levels in vegetables are known to vary considerably according to species, variety and

**Table 1** Nitrate content in fresh vegetables

| Vegetable | Nitrate (mg/100 g) |
|---|---|
| Tahtsai | 631 |
| Spinach | 487 |
| Chingentsai | 383 |
| Pakchoi | 323 |
| Malabar nightshade | 99 |
| Chinese chive | 77 |
| Cabbage | 69 |
| Lettuce | 37 |
| Asparagus bean | 13 |
| Garlic | 12 |

**Table 2** Nitrate content of chingentsai harvested in summer and winter

| | Nitrate (mg/100 g) | |
|---|---|---|
| | summer | winter |
| Leaf | 290 ± 76* | 184 ± 58 |
| Stem | 513 ± 75* | 371 ± 73 |
| Edible portion | 451 ± 66* | 330 ± 65 |

Values are the mean ± SD (n = 5).
Differences between summer and winter were significant (*: $p < 0.05$).

**Table 3** Nitrate content of tahtsai harvested in summer and winter

| | Nitrate (mg/100 g) | |
|---|---|---|
| | summer | winter |
| Leaf | 298 ± 103* | 159 ± 82 |
| Stem | 783 ± 123* | 609 ± 116 |
| Edible portion | 605 ± 127* | 457 ± 111 |

Values are the mean ± SD (n = 5).
Differences between summer and winter were significant (*: $p < 0.05$).

growing conditions [13]. A higher nitrate level was observed in greenish-yellow leaf vegetables [5]. Generally, a lower level of nitrate was observed in cucumbers and tomatoes (below 50 mg/100 g), while higher levels were observed in lettuce, spinach, radish and Chinese cabbage [14].

**Table 4** Nitrate content of chingentsai grown by hydroponics and soil culture

| Nitrate (mg/100 g) | |
|---|---|
| hydroponics | soil cultute |
| 581 ± 165** | 371 ± 84 |

Values are the mean ± SD (n = 5).
Differences between summer and winter were significant (**: $p < 0.01$).

Table 2 and 3 show the comparison of nitrate content in chingentsai and tahtsai harvested in summer (July and August 1990) and winter (November 1990 to March 1991) There was a significant difference in the nitrate content in leaf, stem and edible portion of chingentsai between summer and winter ($p < 0.05$), and there was a significant difference in the nitrate content in leaf, stem of tahtsai between summer and winter ($p < 0.05$). Some studies conducted in Europe have shown that nitrate levels of vegetables in winter harvests were higher than those in the summer. However, our results demonstrated contrary findings. This disparity in data may be due to different environmental conditions (i.e. length of exposure to sunlight, cultivation methods and seeding time).

The comparisons of nitrate content in chingentsai grown by hydroponics and in a soil culture are shown in

**Table 5** Nitrate content of chingentsai grown by hydroponics and soil culture

| | Nitrate (mg/100 g) | |
|---|---|---|
| | hydroponics | soil cultute |
| Leaf | 544 ± 224** | 201 ± 86 |
| Stem | 587 ± 154* | 423 ± 93 |

Values are the mean ± SD (n = 5).
Differences between hydroponics and soil culture were significant (*: $p < 0.05$, **: $p < 0.01$).

**Table 6** Effect of cooking on nitrate levels in vegetables

| | Nitrate (mg/100 g) | | residual ratio (%) |
|---|---|---|---|
| | Raw | cooking | |
| Tahtsai | 631 | 419 | 66.4 |
| Spinach | 487 | 201 | 41.3 |
| Chingentsai | 383 | 294 | 76.8 |
| Pakchoi | 323 | 254 | 78.6 |

Values are means before and after cooking (boiling for 3 min. and soaking for 2 min.).

**Table 7** Changes of nitrate level in chingentsai after cooking

| | Nitrate (mg/100 g) | | |
|---|---|---|---|
| | Leaf | Stem | Edible portion |
| Raw | 218.2 ± 79.0 | 420.8 ± 46.9 | 381.5 ± 51.3 |
| Boiling for 1 min. Soaking for 2 min. | 175.3 ± 17.9 | 389.2 ± 30.8 | 349.0 ± 25.3 |
| Boiling for 3 min. Soaking for 2 min. | 95.6 ± 4.2 ** | 328.0 ± 24.6 2 | 246.1 ± 48.4 * |
| Raw | 237.8 ± 73.2 | 423.1 ± 46.7 | 387.5 ± 50.3 |
| Boiling for 1 min. Soaking for 2 min. | 180.7 ± 19.4 | 392.9 ± 33.6 | 353.9 ± 29.3 |
| Boiling for 1 min. Soaking for 10 min. | 139.2 ± 16.2 * | 387.5 ± 21.8 | 342.6 ± 19.4 |
| Raw | 178.0 ± 55.9 | 387.8 ± 17.9 | 343.5 ± 27.2 |
| Boiling for 3 min. Soaking for 2 min. | 89.0 ± 15.9 | 307.0 ± 21.4 ** | 248.0 ± 22.1 ** |
| Boiling for 3 min. Soaking for 10 min. | 78.6 ± 9.5 | 273.6 ± 8.7 | 221.0 ± 5.6 |

Values are the mean ± SD (n = 5).
Significantly different at the level of *: $p < 0.05$ and **: $p < 0.01$.

Tables 4 and 5. There was a significant difference in the nitrate content between hydroponics and soil culture (Table 4, $p < 0.01$). There was also a significant difference in the nitrate content in the leaf ($p < 0.01$) and stem sections ($p < 0.05$) (Table 5).

Table 6 shows the effect of cooking on nitrate levels in some vegetables. The procedure of boiling for 3 min. and soaking for 2 min. tended to lower nitrate content since nitrate is soluble and readily leaches into water. It was found that between 41% and 79% of the nitrate contained in fresh vegetables was lost when they were cooked. This was similar to findings by Pickston et al. [11] and Abo et al. [12].

Table 7 shows the changes of nitrate level in chingentsai after cooking. The level of residual nitrate present in the vegetable was affected significantly by the cooking process ($p < 0.05$, $p < 0.01$). These results indicate that the use of vegetable cooking water for stews, sauces, soups and gravies might be regarded with some caution, especially for babies and young children, achlorhydria cases and other possible susceptible persons.

## References


1. Gangolli SD, Vandenbrandt PA, Feron VJ, Janzowsky C, Koemanan JH, Speijers GJA, Spiegelhalder B, Walker R and Wishnok JS: Assessment of nitrate, nitrite and N-nitroso compouns. Eur. J. Pharmacol. (1994) **292**, 1-38.
2. Schuddeboom LJ: Nitrates and Nitrite in Foodstuffs (Council of Europe Press) Strasbourg (1993) pp 5-14.
3. WHO: Evaluation of Certain Food Additives and Contaminants. 44th Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series No. 859, Ceneva (1995) pp. 29-35.
4. Stephany RW and Schuller PL: Daily dietary intakes of nitrate, nitrite and volatile N-nitrosamines in the Netherlands using the duplicate portion sampling technique. Oncology (1980) **37**, 203-210.
5. WHO: Toxicological Evaluation of Certain Food Additives and Contaminants. WHO Food Additives Series No. 35, Geneva (1996) pp 269-360.
6. Hotchkiss JH, Helser MA, Maragos CM and Weng YM: Nitrate, nitrite, and N-nitroso compounds. Food Safety Assessment, edited by Finley JW, Robinson SF and Armstrong DJ, American Chemical Society, Washington DC (1992) pp 401-418.
7. Petersen A and Stoltze S: Nitrate and nitrite in vegetables on the Danish market: content and intake. Food Addit. Contam. (1999) **16**, 291-299.
8. Vaessen HA and Schothorst RC: The oral nitrate and nitrite intake in the Netherlands: evaluation of the results obtained by PHLC analysis of duplicate 24-hour diet samples collected in 1994. Food Addit. Contam. (1999) **16**, 188-188.
9. Ysart G, Clifford R and Harrison N: Monitoring for nitrate in UK-grown Lettuce and Spinach. Food Addit. Contam. (1999) **16**, 301-306.
10. Knight TM, Foman D, Aldabbagh SA. and Doll R: Estimation of dietary Intake of Nitrate and Nitrite in Great Britain. Food Chem. Toxicol. (1987) **25**, 277-285.
11. Pickston L, Smith JM and Todd M: Nitrate and nitrite levels in fruit and vegetables in New Zealand and the effect of storage and pressure cooking on these levels. Food Techn. in New Zealand (1980) **15**, 11-17.
12. Abo Bakr TM, El-Iraqui SM, Huissen MH: Nitrate and nitrite contents of some fresh and processed Egyptian vegetables. Food Chem. (1986) **19**, 265-275.
13. Bednar CM, Kies C and Carlson M: Nitrate-nitrite levels in commercially processed and home processed beets and spinach. Plant Foods Hum. Nutr. (1991) **41**, 261-268.
14. Chung SY, Kim JS, Kim M, Hong MK, Lee JO, Kim CM and Song IS: Survey of nitrate and nitrite contents of vegetables grown in Korea. Food Addit. Contam. (2003) **20**, 621-628.


Accepted March 31, 2004.