academicJournals

Vol. 3(1), pp. 1-5, September, 2013
DOI: 10.5897/ISABB-JFAS11.033
ISSN 1937-3244 ©2013 Academic Journals
http://www.isabb.academicjournals.org/JFAS

ISABB Journal of Food and Agriculture Science

Full Length Research Paper

Nitrate concentration of leafy vegetables: A survey of nitrite concentrations in retail fresh leafy vegetables from daily markets of different locations

J. C. Jana* and P. Moktan

Department of Vegetable and Spice Crops, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, PIN- 736165 West Bengal, India.

Accepted 12 March 2012

In this study, nitrate content in three leafy vegetables namely radish, palak and amaranth were studied after a survey from four different markets at different elevations namely Gangtok of Sikkim State, Kalimpong, Siliguri and Cooch Behar of West Bengal. Average nitrate content of radish leaves (1521.13 mgkg^{-1}) was found to be maximum at Kalimpong market followed by Gangtok, Siliguri and Cooch Behar markets, whereas average nitrate contents of palak (2971.26 mgkg^{-1}) and amaranth leaves (1854.96 mgkg^{-1}) were maximum at Gangtok market followed by Kalimpong, Siliguri and Cooch Behar markets. Among the samples of the three leafy vegetables, nitrate content was higher than the Acceptable Daily Intake (ADI) with an average of 60 kg. If a person consumes 100 g per day in some palak samples collected from all survey markets, this incidence was maximal at Gangtok. Therefore, more care is needed for nitrogen fertilization in palak crop not to exceed the leaf nitrate content over the ADI limit.

Key words: Nitrate concentration, leafy vegetables.

INTRODUCTION

Nitrate is a naturally occurring form of nitrogen and is an integral part of nitrogen cycle in the environment. Nitrate is usually formed from fertilizers, decaying plants, manure and other organic residues. It has been found that due to the increased use of synthetic nitrogen fertilizers and livestock manure in the intensive agriculture, vegetable and drinking water may contain higher concentrations of nitrate than was found in the past. Exposure of humans to nitrate is mainly exogenous which occurs mainly through the consumption of vegetables, and to a lesser extent water and other foods. More than three quarter of our average nitrate intake comes from vegetables, which provide about 80% of the average daily dietary intake. In evaluating nitrate and nitrite, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) set the Acceptable Daily Intake (ADI) for nitrate at 0 to 3.65 mgkg^{-1} body weight. Subsequently, the European Communities' Scientific Committee for Food (SCF, 1997) also set an ADI for nitrate of 0 to 3.65 mgkg^{-1} body weight. Compared with this ADI, the ingestion of only 100 g of raw vegetables with a nitrate concentration of 2190 mgkg^{-1} fresh matter (FM) corresponds to the whole nitrate ADI for a person of 60 kg though, it must be observed that some components of vegetables (for example, ascorbic acid and phenols etc) have been reported to inhibit the toxic effects of nitrites (Santamaria, 2006). Leafy vegetables grown under different agro-ecological conditions accumulate nitrate to potentially harmful concentrations. Generally, nitrate accumulating vegetables belong to the families Brassicaceae (rocket, radish and mustard), Chenopodiaceae (beetroot, Swiss chard and spinach), Amaranthaceae (Amaranthus), Asteraceae (lettuce) and Apiaceae (celery and parsley). Nitrate content can vary also within species, cultivars and even genotypes with

*Corresponding author. E-mail: janajc@rediffmail.com.

different ploidy. Nitrate content differs in the various parts of a plant. Indeed, the vegetable organs can be listed by decreasing nitrate content as follows: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed (Maynard et al., 1976; Santamaria et al., 1999). The nitrate content of vegetables can be affected by processing of the food, the use of fertilizers and growing conditions, especially the soil temperature and (day) light intensity (Gangolli et al., 1994). Vegetables such as beetroot, lettuce, radish, and spinach often contain nitrate concentrations above 2500 mg/kg, especially, when they are cultivated in greenhouses. The differing capacities to accumulate nitrate can be correlated with differing location of the nitrate reductase activity, as well as, to differing degree of nitrate absorption and transfer in the plant. An important part of the vegetable nutrition research has been directed towards efficient nitrogen use for high yields and quality together with minimal nitrate concentrations in the harvested commodity.

In view of the importance of vegetables to human health and fact that many people have now resulted to eating of vegetables for the well being of their health, the aim of this study is therefore to determine nitrate concentrations in some common leafy vegetables with the following objectives:

1. To demonstrate ranges of nitrate content of some common leafy vegetables namely radish, palak and amaranth sold in different markets of different locations that is, North Eastern hills of Sikkim and West Bengal and Terai region of West Bengal.
2. To assess the relative safety of some common leafy vegetables based on European Standard (EU) nitrate limits.

MATERIALS AND METHODS

Sampling

A sampling method which factors in type of vegetable (Radish, Palak and Amaranth) and markets with different locations (Gangtok of Sikkim State located between East 88° 03' 40” to 88° 51' 9” longitude and North 27° 03' 47” to 28° 07' 34” latitude, Kalimpong Darjeeling district of West Bengal located at latitude of 27.06° North to longitude of 88.47° East, Siliguri of Darjeeling district of West Bengal located at latitude of 26° 42' North and longitude of 88° 25' East and Cooch Behar of West Bengal located at latitude of 26° 20' North and at longitude of 89° 29' East) was established (Table 1). Sampling was replicated thrice within each market at intervals of two weeks with three different brands (sellers).

Pre-treatment and nitrate analysis

All sub-samples were put into cooler boxes immediately after purchase and brought to the Post Graduate Laboratory, Department of Vegetable and Spice Crops, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar. As needed, sub-samples were washed to remove soil and blotted on paper. Dead leaves and non-edible parts were removed and weighed.

The amount of nitrate in different samples was estimated according to rapid colorimetric determination of nitrate in plant tissue by the nitration of salicylic acid as reported by Cataldo et al. (1975). This method is based on forming a nitro derivative of salicylic acid with nitrate. High (sub-milimolar) concentrations of nitrite could also form a nitro derivative with salicylic acid. Thus, nitrite should be removed from the sample. In the first step of the analysis, a saturated solution of sulfamic acid is added to the sample to prevent interference from nitrite. Sulfamic acid converts nitrite to nitrogen gas. Secondly, salicylic acid reacts with nitrate under acidic conditions to form nitrosalicylic acid. The the pH was brought to above 12 to form a chromophore with a maximum absorbance at 410 to 420 nm.

Standards

The following standards were applied in this study:

- Stock solution 0.25 g/L NO_3^-N (= 250 mg/L, 250 µg/mL).
- In a 1.0 L standard flask containing approximately 600 ml Type-I-water, 1.805 g potassium nitrate was dissolved.
- It was ensured that all potassium nitrate (KNO_3) is dissolved, made up to the mark with Type-I-water (Table 2), mixed and stored in a suitably labelled plastic container.

Blanks

A blank of 0.25 ml extractant (or H_2O) with the normal reagents was normally sufficient. For pigmented samples a separate blank was required and prepared for each sample. This blank consisted of the extract, 0.8 ml of concentrated sulphuric acid (minus salicylic acid) and 19 ml of 2 N NaOH.

Chemicals

NO_3^- Reagent A: Saturated solution of amidosulfonic (sulfamic) acid
NO_3^- Reagent B: 5% (w/v) salicylic (2-hydroxybenzoic) acid in pure H_2SO_4
NO_3^- Reagent C: 4 M NaOH

Procedure

Before measuring the nitrate concentration in the sample, we made sure to dilute it to the above mentioned range.
1. 10 µl reagent A was added to cuvette (4 ml macro-cuvette)
2. 40 µl of sample was added on reagent A (pipette to mix reagent A and the sample)
3. 200 µl reagent B was added
4. Waited for 10 min
5. 2 ml reagent C was added
6. Waited for 20 min
7. Mixed with a clean pipette tip to remove the formed bubbles
8. Absorbance was read at 420 nm
9. The liquid was discharged to sink

The procedure was done with water instead of the sample for the “blank” measurement.

Calibration and notes

Stock solutions were made of 1 to 5 mM, solutions B and C were cold and solution B was kept in the dark. Nitrate content was expressed as milligrams nitrate per kilograms on a fresh weight basis (mg NO3 /kg FW).

Table 1. Sampling method for market survey.

Factor	Level
Type of vegetables (3)	Radish, Palak, Amaranth
Market (4)	Three different markets of different elevations
Interval (2)	Intervals of two weeks
Brand (3)	Three different sellers per vegetables
Sample (3)	Three sub-samples per brand
Total number	3 × 4 × 2 × 3 × 3 = 216 samples

Table 2. Prepare standards containing approximately 0 to 60 µg NO_3^-N in a 0.25 ml aliquot.

Amount of NO_3^-N	Volume of stock (ml)	Volume of extractant (or H_2O)
62.5	0.25	0.00
50.0	0.20	0.05
37.5	0.15	0.10
25.0	0.10	0.15
12.5	0.05	0.20
0.00	0.00	0.25

Statistical analysis

The data collected were analyzed using Fisher's analysis of variance technique and differences among the various treatments were determined by using least significant difference test at 5% probability level (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

Nitrate concentrations in leafy vegetables procured from different markets are summarized in Table 3 for radish leaves, Table 4 for palak leaves and Table 5 for amaranth leaves. The SCF has set ADI for nitrate ion on the basis of body weight of the consumer that is, 3.65 $mgkg^{-1}$ body wt day^{-1}.

Nitrate concentration in radish (*Raphanus sativus* L.) leaves

Nitrate concentrations in fresh radish leaves procured from different markets are summarized in Table 3. The nitrate content ranged from 951.25 to 1847.58 $mgkg^{-1}$ fresh weight of the sample. In this study, the average nitrate content of radish leaves was found to be highest at Kalimpong market with a value of 1521.13 $mgkg^{-1}$ which was followed by Gangtok market at 1196.01 $mgkg^{-1}$ and Siliguri market at 1248.14 $mgkg^{-1}$. The average nitrate content of 882.17 $mgkg^{-1}$ was found to be the minimum at Cooch Behar market.

The nitrate content of the sample procured from Kalimpong showed an increase of 72.43% over the samples of Cooch Behar market. However, in all the four markets, the nitrate content in radish leaves did not cross the ADI limit of 3.65 $mgkg^{-1}$ body wt day^{-1} set by the Scientific Committee for Food (SCF).

Nitrate concentration of palak (*Beta vulgaris* var. *bengalensis Hort*.) leaves

The nitrate concentration of palak leaves procured from different markets are summarized in Table 4 and from the data in the table, we found that the average nitrate concentration was in the range from 783.56 to 3731.53 $mgkg^{-1}$, respectively. The maximum nitrate concentration of 2971.26 $mgkg^{-1}$ was found to be in the sample procured from the Gangtok market followed by the sample procured from Kalimpong market at 2523.99 $mgkg^{-1}$ and Siliguri market at 2405.20 $mgkg^{-1}$. The mini-mum nitrate concentration of 1888.70 $mgkg^{-1}$ was found to be in the sample procured from the Cooch Behar market. The nitrate content of the sample procured from Gangtok market showed an increase of 57.32% over the sample of Cooch Behar market.

Vegetable nitrate content was higher than the ADI for an average 60 kg person if consumed 100 g per day in some Palak samples mostly when it is collected from Gangtok. It may be due to a complex interaction between the growing season, production method and location in relation to the amount of sunlight. In low light intensity and low temperature condition, the accumulated nitrate is not rapidly reduced as in other places with high temperature and bright sunshine.

It has also been reported that accumulation of large amounts of nitrate in palak may be due to their low Nitrate Reductase Activity (NRA), as evident by the negative relationship. Other causal factors may be related to variations in the uptake and distribution of nitrate needed

Table 3. Nitrate content of radish leaves collected from different markets.

Market	Range ($mgkg^{-1}$)	Average ($mgkg^{-1}$)
Gangtok	951.25 – 1567.21	1196.01
Kalimpong	975.25 – 1749.23	1521.13
Siliguri	894.72 – 1847.58	1249.14
Cooch Behar	676.55 – 1121.23	882.17

Table 4. Nitrate content of palak leaves collected from different markets.

Market	Range ($mgkg^{-1}$)	Average ($mgkg^{-1}$)
Gangtok	1984.95 – 3625.22	2971.26
Kalimpong	1184.57 – 3545.67	2523.99
Siliguri	1757.72 – 3414.79	2405.20
Cooch Behar	783.56 – 3731.53	1888.70

Table 5. Nitrate content of amaranth leaves collected from different markets.

Market	Range ($mgkg^{-1}$)	Average ($mgkg^{-1}$)
Gangtok	1243.67 – 2625.52	1854.96
Kalimpong	1021.42 – 1598.21	1330.84
Siliguri	884.63 – 1578.84	1233.95
Cooch Behar	690.93 – 1784.95	1176.89

for NRA, differences in generation of electron donors needed in the assimilative pathway (Cantliffe, 1973) in photosynthetic capacity (Behr and Wiebe, 1992) or ability to generate and translocate respiratory substrate and reducing equivalents.

Nitrate concentration of amaranth (*Amaranthus* spp.) leaves

The data presented in Table 5 showed the average nitrate concentrations in the range of 690.93 to 2625.52 $mgkg^{-1}$. Among the four markets, the maximum recorded average nitrate concentration of 1854.96 $mgkg^{-1}$ originated from the sample procured from the Gangtok market. This was then followed by the Kalimpong market at 1330.84 $mgkg^{-1}$ and by the Siliguri market at 1233.95 $mgkg^{-1}$. The minimum average nitrate concentration of 1176.89 $mgkg^{-1}$ was found to be in the sample procured from Cooch Behar market.

The nitrate content of the sample procured from Gangtok market showed an increase of 57.62% over the sample of Cooch Behar market. However, in all the four markets, the nitrate content in radish leaves did not cross the ADI limit of 3.65 $mgkg^{-1}$ body wt day^{-1} set by the Scientific Committee for Food (SCF, 1997). Variation in nitrate content between plant species and even between cultivars of the same species has been reported earlier by Blom-Zandstra and Eenink, (1986) and Reinink et al. (1994). Over expression of NR genes may be a useful approach to reduce the nitrate content of plants that have a propensity to accumulate the same and to improve their quality for human consumption, although, genetic manipulation of activities of nitrate assimilatory enzymes may not increase yield and/or nitrogen use efficiency of plants (Quillere et al., 1994). Nitrate consumption through vegetables can be kept low by harvesting them at the proper time. Some earlier reports have advised that spinach crop should be harvested in the afternoon of a sunny day when nitrate concentration in the leaves is low (Steingrover et al., 1982; Reinink, 1991). Thus a careful selection of vegetable genotypes based on the relationship between nitrate and Nitrate Reductase Activity (NRA), coupled with due management of the nutrition and harvest regime helps to avoid nitrate accumulation and the associated health hazards. Moreover, by cooking vegetables in water (with low nitrate concentration), at least 50% of accumulated nitrate can be removed (Meah et al., 1994).

Conclusion

Average nitrate content of radish leaves (1521.13 $mgkg^{-1}$) was found to be maximum at Kalimpong market followed by Gangtok, Siliguri and Cooch Behar markets, whereas, average nitrate contents of palak leaves (2971.26 $mgkg^{-1}$) and amaranth leaves (1854.96 $mgkg^{-1}$) were maximum at Gangtok market followed by Kalimpong, Siliguri and

Cooch Behar markets. Among the samples of the three leafy vegetables, nitrate content was higher than the ADI for an average 60 kg person if consumed 100 g per day in some palak samples collected from all survey markets and this incidence was maximum at Gangtok. Therefore, more care should be taken for nitrogen fertilization in palak crop so as not to exceed the leaf nitrate content over the ADI limit.

REFERENCES

Behr U, Wiebe HJ (1992). Relation between photosynthesis and nitrate content of lettuce cultivars. Sci. Hortic., 49:175-179.

Blom Zandstra M, Eenink AH (1986). Nitrate concentration and reduction in different genotypes of lettuce. J. Am. Soc. Hortic. Sci., 111:908-911.

Cantliffe DJ (1973). Nitrate accumulation in table beets and spinach as affected by nitrogen, phosphorus, and potassium nutrition and light intensity. Agron. J., 65:563-565.

Cataldo DA, Haroom M, Schrader LE, Young VL (1975). Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. Comm. Soil Sci. Plant Anal. 6(1):71-80.

Gangolli SD, Van Den Brandt P, Feron V, Janzowsky C, Koeman J, Speijers G, Spiegelhalder B, Walker R, Winshnok J (1994). Assesment of nitrate, nitrite and N-nitroso compounds. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect., 292:1-38.

Maynard DN, Barker AV, Minotti PL, Peck NH (1976). “Nitrate accumulation in vegetables.” Advances in Agronomy, 28:71-118.

Meah MN, Harrison N, Davies A (1994). Nitrate and nitrite in foods and the diet. Food Addit. Contam., 11:519- 532.

Quillere L, Dufoss C, Roux Y, Foyer CH, Caboche N, Marot-Gaudry JF (1994). The effect of deregulation of NR gene expression on growth and nitrogen metabolism of Nicotiana plumbaginifolia plants. J. Exp. Bot., 45:1205-1211.

Reinink K, Vannes M, Groenwold R (1994). Genetic variation for nitrate content between cultivars of endive. Euphytica, 75:41- 48.

Reinink K (1991). Genotype X Environment interaction for nitrate concentration in lettuce. Plant Breed, 107:39–49.

Santamaria P, Elia A, Serio F, Todaro E (1999). A survey of nitrate and oxalate content in retail fresh vegetables. J. Sci. Food Agri., 79:1882–1888.

Santamaria P (2006). Nitrate in Vegetables: Toxicity Content, Intake and European Commission Regulation. J. Sci. Food Agron., 86: 10-17.

SCF (Scientific Committee on Food) (1997). Assessment of dietary intake of nitrates by the population in the European Union, as a consequence of the consumption of vegetables, in Reports on tasks for scientific cooperation: Report of experts participating in Task 3.2.3, ed by European Commission, Brussels, p. 34.

Steel RG, Torrie JH (1984). Principles and Procedures of Statistics: A Biometrical Approach. Mc Graw Hill Co. New York. pp. 173 – 191.

Steingrover E, Oosterhius R, Wieringa F (1982). Effect of light treatment and nutrition on nitrate accumulation in spinach (*Spinacia oleracea* L.). Z. Pflanzenphysiol., 107:97–102.