The Nitrate Content in Some Green Leafy Vegetables with Different Cultivation Methods in Thailand

Chatchada Petpiamsiri[*] *Tippawan Siritientong*[*] *Kaew Kangsadalampai*[*] *Linna Tongyonk*[*]

ABSTRACT

High nitrate food products were reported to increase risk of cancer and many diseases. Vegetables were the main sources of nitrate intake in human. The objective of this study was to investigate nitrate contents (NO_3-N) of four green leafy vegetables, namely Chinese kale (*Brassica oleracea* var. *alboglabra*), Chinese mustard (*Brassica rapa* var. *parachinensis*), Chinese cabbage (*Brassica rapa* var. *pekinensis*), and Bok choy (*Brassica rapa* var. *chinensis*) grown in four different cultivation methods (conventional, hygienic, organic, and hydroponic methods). The nitrate contents (NO_3-N) were analyzed by AOAC brucine colorimetric method between November, 2017 and February, 2018. Chinese kale, Chinese mustard, and Chinese cabbage were cultivated with hydroponic method that contained higher nitrate compared to that of the other cultivation methods ($p < 0.05$). Bok choy cultivated with conventional method revealed statistically higher nitrate content than that of vegetables cultivated with other methods ($p < 0.05$). Only hydroponic Chinese mustard ($3,627 \pm 900$ mg/kg) and conventional Bok choy ($3,537 \pm 817$ mg/kg) had nitrate content exceeded the EU standard of 3,500 mg/kg. Among the four cultivation methods, hydroponics had the significant highest average nitrate amount ($2,994 \pm 958$ mg/kg) ($p < 0.05$). With respect to types of vegetables, Bok choy contained the significant highest average nitrate amount ($2,634 \pm 1,071$ mg/kg) ($p < 0.05$). Since both cultivation method and type of vegetable affect nitrate accumulation of green leafy vegetables, consuming various vegetables in each meal may help to reduce the risk of over-exposure of nitrate to human.

Keywords: nitrate accumulation, green leafy vegetable, organic, hygienic, hydroponics, conventional

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Correspondence: Linna Tongyonk. Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330. THAILAND. E-mail: linna.t@chula.ac.th

[*] Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Introduction

Nitrogen, an important component necessary to produce chlorophyll, protein, and genetic materials is an essential nutrient for the growing of plants. One of the main sources of nitrogen taken up by plants or vegetable is come from nitrate containing fertilizer. Providing an excess of this fertilizer may result in nitrate accumulation in vegetables[1, 2]. In human daily diet, vegetables are recognized as an important source of nitrate intake. By estimation, a daily amount concentration of nitrate consumed by man is approximately 75-100 mg, of which almost 80% come from vegetables[3-5]. Such details have raised an important concern because high level of nitrate can be converted to nitrite in human body by the action of bacteria as well as mammalian enzymes likes nitrate reductase found in saliva and gastric juice[4, 6]. This nitrite may interact with amino acids, amides, and amines to form N-nitroso compounds. Although nitrate is not quite toxic, its products such as nitrite and N-nitroso compounds are known to be carcinogenic[4, 6]. Together with the previous studies showing that the plasma level of nitrate is correlated with the risk of several cancers including gastric[7], prostate cancer[5] and bladder cancer[8], thus the level of accumulated nitrate in dietary vegetables should be precisely monitored.

Recently, there is a dramatic shift in healthy food business and the key components of healthy foods are vegetables. There is a context of hypothesis that the amount of nitrate found in vegetables may vary depending on vegetable species, harvesting time, light intensity, ambient temperature, nitrogen fertilization, and the cultivation methods[1], however, according to the previous report indicating that the amount of nitrate in soil is the major factor determining the nitrate content in vegetables[2]. The hypothesis of this study was that the cultivation method should be among the most impacting factor determining nitrate level in vegetable products. Regarding vegetable cultivation methods, there are four cultivation methods commonly employed including conventional, hygienic, hydroponic, and organic. In conventional cultivation, the vegetables are grown in soil, using both of chemical fertilizers and pesticides[9]. Hygienic cultivation is essentially similar to the conventional culture; however, hygienic cultivation uses the short half-life chemicals and terminates the chemical for detoxified period before harvesting[9]. In hydroponic cultivation, vegetables are grown in the water supplemented with the suitable nutrients and fertilizer[9]. The organic cultivation method cultivates vegetables in soil using natural fertilizers and avoiding chemicals[9].

As the types of vegetables and cultivation methods may vary the nitrate residues, this study is, therefore, aimed at determining the

nitrates contents in different types of vegetables; Chinese kale (Thai name: Pak-ka-na), Chinese mustard (Thai name: Pak-kwang-tung) and Chinese cabbage (Thai name: Pak-kad-kao) and Bok choy (Thai name: Pak-kwang-tung-hong-tae) which were obtained from four different cultivation methods as mentioned previously.

Materials and Methods

Samples

Four types of green leafy vegetables, including Chinese kale (*Brassica oleracea* var. *alboglabra*), Chinese mustard (*Brassica rapa* var. *parachinensis*), Chinese cabbage (*Brassica rapa* var. *pekinensis*) and Bok choy (*Brassica rapa* var. *chinensis*) in four cultivation types (organic, hygienic, hydroponics, and conventional) were purchased from different sources. Conventional vegetables grown in soil using both chemical fertilizers and chemical pesticides were purchased from fresh local market in Bangkok. Hygienic vegetables with "Q mark" label grown in soil using the short half-life chemicals were purchased from supermarket in Bangkok. The "Q mark" guaranteed production of hygienic vegetables of good agricultural practice (GAP) standard which certified by national bureau of agricultural commodity and food standards (ACFS)[9]. Organic vegetables with "Organic Thailand" label grown in soil using natural fertilizers and natural pesticides were purchased from supermarket in Bangkok. The "Organic Thailand" label assured production of organic vegetables which certified by department of agriculture[9]. Hydroponic vegetables grown with the suitable nutrient solution were acquired directly from hydroponic farm in Sukhothai province, Thailand. Total numbers of samples were 16 samples from 4 types of vegetables with 4 different cultivation methods. Each sample was collected between November, 2017 and February, 2018 in different three batches and was purchased in 300 to 500 grams quantities per batch.

Sample preparation

According to AOAC method 976.14[10] with modifications was employed for vegetable extraction. Each fresh vegetable was washed with flowing water, and dried thoroughly with adsorbent paper trays. After removing the non-edible part, the edible part of vegetable was blended to be fine pieces. Each 50 g of fine sample was added with 200 mL deionized water then homogenized with Ultra-turrax T25 (IKA®-Werke GmbH & Co. KG, Germany) for 5 min. The mixture was boiled on hot water bath (70-80 °C) for 15 min. When the mixture was cool, the solid insoluble part was separated by filtration through Whatman No.1 with Buchner funnel. The final volume of filtrate was adjusted to 200 mL with deionized water and this extracted sample was kept in a 4 °C for nitrate content analyzing within 12 hours.

Nitrate determination

Nitrate determination was achieved according to AOAC official method 973.50: brucine colorimetric method. The brucine method is based on nitrate determination through the yellow compound from the nitrate ion reacts with brucine-sulfanillic acid reagent in acidic (H_2SO_4) and high temperature condition (100 °C)[11]. For each determination, three replicates were performed. Absorbance of the yellow compound was read at 410 nm against a reagent blank by using a spectrophotometer (ThermoSpectronic, Becthai Bangkok Equipment & Chemical Co.,Ltd., Thailand). Nitrate content expressed as nitrate-nitrogen (NO_3-N) mg/L was calculated from the linear regression equation of the standard curve which was performed in each experiment. The results were continuously interpreted in NO_3-N mg/kg of fresh vegetable weight. Total nitrate values were 9 values from three replicate of three different batches of each sample.

Statistical analysis

Data were presented as means ± SD of triplicate experiments. The statistical tests were performed by using IBM SPSS Statistics 22 (IBM, Inc.). Comparison of the nitrate contents in four leafy vegetables with four different cultivation methods were performed by one-way ANOVA and post-hoc test. The significance of difference was defined at $p < 0.05$.

Results

The average nitrate contents of four vegetable samples, namely Chinese kale, Chinese mustard, Chinese cabbage, and Bok choy cultivated with four cultivation methods (organic, hydroponics, hygienic, and conventional) are shown in Table 1. When considering the effect of cultivation method on nitrate level in the same type of vegetable, it was found that three of the four types of selected vegetables, including Chinese kale, Chinese mustard, and Chinese cabbage which cultivated by hydroponic method showed higher nitrate content than those cultivated by other methods with statistically significant. While no significant differences were observed in nitrate content of Bok choy grown with the cultivation of hydroponics, hygienic, and conventional methods, it was apparent that the nitrate level of organic Bok choy was lower than conventional Bok choy. In addition, the nitrate contents of samples were compared among each type of vegetable which cultivated by the same cultivation method. By organic method there were no significant differences in nitrate level in four types of plants. By hygienic method, Bok choy contained the highest average nitrate level, similar to what was found in conventional growing vegetables.

Table 1 Nitrate Contents in Chinese kale, Chinese mustard, Chinese cabbage and Bok choy Produced by Different Cultivations; Organic, Hygienic, Hydroponic, and Conventional Methods.

	NO_3-N content of fresh weight (mg/kg)Average ± SD (range)			
Type of vegetable	Organic	Hygienic	Hydroponics	Conventional
Chinese kale	1,906 ± 454$^{a\ \dagger}$	1,269 ± 912$^{a\ \dagger}$	2,757 ± 462$^{b\ \dagger\ddagger}$	1,883 ± 361$^{a\ \dagger}$
(Pak-ka-na)	(1,413-2,400)	(530-2,413)	(2,558-3,049)	(1,512-2,132)
Chinese mustard	1,912 ± 1,391$^{a\ \dagger}$	1,289 ± 1,066$^{a\ \dagger}$	3,627 ± 900$^{b\ \dagger}$	1,500 ± 770$^{a\ \dagger}$
(Pak-kwang-tung)	(194-2,905)	(573-2,695)	(2,797-4,386)	(499-2,104)
Chinese cabbage	1,363 ± 739$^{a\ \dagger}$	953 ± 383$^{a\ \dagger}$	3,211 ± 633$^{b\ \dagger\ddagger}$	1,409 ± 900$^{a\ \dagger}$
(Pak-kad-kao)	(661-2,313)	(571-1,391)	(2,687-3,674)	(624-2,538)
Bok choy	2,007 ± 726$^{a\ \dagger}$	2,609 ± 887$^{ab\ \ddagger}$	2,382 ± 1,266$^{ab\ \ddagger}$	3,537 ± 817$^{b\ \ddagger}$
(Pak-kwang-tung-hong-tae)	(1,326-2,927)	(2,210-3,458)	(731-3,302)	(2,925-4,577)

Note: Number of samples (*n*) = 9 (one sample had 9 values, triplicate nitrate values from three different batches)

Different letters (a, b) indicate the difference ($p < 0.05$) between cultivation methods of each vegetable

Different symbols (†, ‡) indicate the difference ($p < 0.05$) between types of vegetables of each cultivation

The distribution of nitrate content values of vegetable grouping by vegetable types and cultivated methods is shown in Figure 1. Figure 1A exhibits the 36 values of nitrate content for each type of vegetable collected from four different cultivation methods. The mean value calculated from the nitrate contents of these 36 samples was also determined. According to type of vegetables, Bok choy had the highest average nitrate content (2,634 ± 1071 mg/kg) ($p < 0.05$) but no significant differences were observed in average nitrate content among Chinese mustard, Chinese kale, and Chinese cabbage. Plot of nitrate contents of vegetable vs. cultivation method is shown in figure 1B. Each cultivation method consisted of 36 values of nitrate contents from four types of vegetables. The data showed that vegetables grown by hydroponic method had the highest average nitrate content (2,994 ± 958 mg/kg) ($p < 0.05$) compared to the mean values obtained from those of other methods.

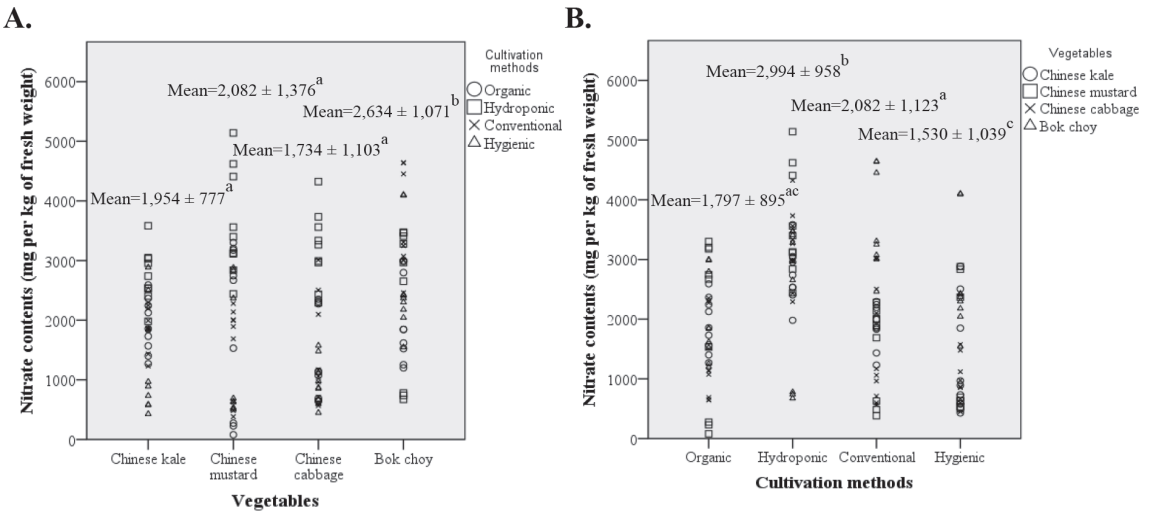

Figure 1 The Distribution of Nitrate Content of All Vegetables Varied in (A) Type of Vegetable and (B) Cultivation Method

Note: Each column of graph was plotted from the single nitrate value of all different samples (*n* = 36, one samples had 9 values from 4 types of vegetables or 4 methods of cultivations)

Mean of nitrate contents were represented in mg/kg of fresh weight, different letters (a-c) indicated the mean difference ($p < 0.05$)

The nitrate content in one portion of serving (80 g) of each fresh vegetable[12] was determined and the data are shown in Table 2. For information on decision making for consumer, the prices per kilogram of each vegetable were also calculated. The results were shown in brackets in Table 2. It was found that vegetables grown by hydroponic method showed a tendency to have high nitrate contents.

Table 2 Estimated Amounts of Nitrate Content (mg) in One Serving (80 g) of Each Vegetable Consumption[12] of Selected Vegetables with Different Cultivations.

Type of vegetable	Nitrate content of fresh vegetable weight[a] (price:baht/kg)			
	Organic	Hygienic	Hydroponics	Conventional
Chinese kale (Pak-ka-na)	153 (200)	102 (152)	219 (400)	151 (63)
Chinese mustard (Pak-kwang-tung)	153 (178)	103 (112)	290 (400)	120 (50)
Chinese cabbage (Pak-kad-kao)	109 (200)	76 (92)	257 (400)	113 (40)
Bok choy (Pak-kwang-tung-hong-tae)	161 (220)	209 (193)	191 (400)	283 (150)

Acceptable Daily Intake (ADI) of nitrate (3.7 mg/kg/body weight/day, expressed as nitrate ion)[18]
ADI of nitrate for Thai male = 255 mg/day and Thai female = 211 mg/day (based on the average weight of Thai 69-kg male adult and Thai 57-kg female adult)[19]
[a] 1 serving of vegetable = 80 g[12]

Discussion

There is no standard set for nitrate content in Thailand. The results of this study, so comparing with the legal limit established by European Union (EU) legislation of fresh spinach which is not exceed 3,500 NO_3-N mg/kg[13]. The nitrate content higher than EU standard value was found in Chinese mustard planting by hydroponic method. Wang and Li[1] also found that mustard family vegetables tended to have high levels of nitrate. Bok choy growing by conventional method had nitrate content slightly higher than the standard value. However, the amounts of nitrate in most vegetable samples in this study were not exceed the limits set by the EU. The result is consistent with the study of Prasad and Chetty[14]. They found that nitrate content in cabbage in Fiji was $2,487 \pm 381$ NO_3-N mg/kg[14]. Kale in Croatia in the autumn was $1,182 \pm 700$ NO_3-N mg/kg[15].

Considering the mean value of nitrate content in each vegetable regardless of cultivation method, it was found that Bok choy had the highest nitrate content among four vegetable samples. The different parts of a plant are the one factor that affects the nitrate accumulation in vegetable. Some studies showed that the nitrate concentration in petiole was higher than in leaf[1, 3]. The characteristic of Bok choy has larger and thicker petioles than other vegetables while other vegetables have large leaves but small petioles. This is probably the reason why bok choy had higher nitrate content than other vegetables.

Normally, roots of vegetables absorb nitrogen from the fertilizer to produce the amino acids in the form of nitrate ions. Nitrate accumulation in vegetables occurs when vegetables absorb nitrate more than they require for their sustainable growth[14]. The maximum mean value of nitrate content was found in vegetables grown by hydroponic method. In hydroponic cultivation, vegetables are grown in the water supplemented with the suitable nutrients and fertilizer[9]. With this characteristic the roots of the plant are exposed to a chemical solution directly and excessive substances may be absorbed by the roots and accumulated in the body of plant[14]. Phupaibul[16] also found that the average amount of nitrate in hydroponic Chinese kale in Thailand had the highest nitrate content 4,529.3 NO_3-N mg/kg among their samples. Nowadays, there are no studies about the suitable amount of nitrate that should be added into hydroponic fertilizer to minimize the accumulation of nitrate in vegetables. In conventional cultivation, vegetables were cultivated in soil cultures and farmers used chemical fertilizers. Nitrate nitrogen of chemical fertilizer dissolves easily in water and moves quickly in soils[17]. Concentrated nitrate ions of chemical fertilizer are easily absorbed by vegetables. Organic fertilizer requires bacteria in soil to convert ammonia into nitrate ions for absorption by vegetables. Therefore, the exact amount of nitrate content in this type of fertilizer cannot be measured[17]. As the result of this study, the vegetables grown in conventional cultivation had higher nitrate content than that of the organic vegetables. Although farmers used chemical fertilizers in hygienic cultivation as same as in conventional cultivation, farmers stopped using the chemicals during pre-harvest period[9]. Therefore, it is possible that hygienic vegetables may be able to eliminate nitrate out during this pre-harvest period. This may cause hygienic vegetables had less nitrate contents than those of grown with other cultivation methods.

In this study all vegetable samples were found to contain nitrate residue in different quantities and some vegetables had nitrate contents higher than standard level of EU. Obtaining nitrate from eating a lot of vegetables may cause adverse health effects. Acceptable daily intake (ADI) of nitrate (3.7 mg/kg body weight/day, expressed as nitrate ion) was reported by the Joint FAO/WHO Expert Committee on Food Additives[18]. Based on the average weight of Thai populations, 69-kg for male and 57-kg for female[19], thus the maximum safety daily level of nitrate consumptions for Thai males and females are 255 mg and 211 mg, respectively. According to the present study, the amount of nitrate in 1 serving or 80 g of vegetables were calculated. This one serving of vegetable

that is equivalent to 80 g is calculated by the National Health Service (NHS) choices[12]. One serving of hydroponic Chinese mustard or hydroponic Chinese cabbage or conventional Bok choy will result in excess nitrate intake over ADI. An appropriate practice to reduce daily consumption of nitrate intake should be performed by incorporation of various vegetables into meals in order to reach the 5 servings or 400 g per day as healthy portions of vegetables recommended by the Food-based dietary guidelines Thailand[20]. The consumption of high nitrate green leafy vegetables along with other low-nitrate vegetables may help to reduce the risk of over-exposure of nitrate. Literature review shows that vegetables with low-nitrate levels are easily found, for instance, asparagus, garlic, onion, potato, mushroom, tomato, broccoli, cucumber and carrot[3, 6]. Another factor that should be concerned is the price. If considered only the price, regardless of the chemical residues, hydroponic vegetables are the most expensive and conventional vegetables are the cheapest.

Foods with high nitrate levels, such as sausages, can cause toxic effects on human health; similarly, vegetables with high nitrate levels may be likely to have some impact on human health as well. Especially in the group of people who are vegetarians, eating plenty vegetables is more likely to be at risk of toxicity of nitrate than normal people. However, green leafy vegetables also contain many vitamins and antioxidants that help to reduce the incidence of human cancer, such as vitamin C. Vitamin C can reduce nitrite before forming a nitrosamines and isothiocyanates that were found as a natural substance in green cruciferous vegetables. Isothiocyanates can inhibit DNA methylation and early and late stage of carcinogenesis[21]. Sulforaphane is the most potent chemopreventive agent among isothiocyanates that can prevent cancer from many mechanisms such as anti-inflammatory or pro-apoptotic[22]. Vegetables are processed by fermentation and boiling that reduces the amount of nitrate accumulated in vegetables[5, 14].

Conclusions

The findings showed that the green leafy vegetables grown with hydroponic method seemed to accumulate higher nitrate in the vegetables than those grown in soil. According to types of vegetables, Bok choy had the highest average nitrate content. Thus, if consumer would like to eat vegetables with high nitrate accumulation, they should also consume other low-nitrate vegetables within the same meal to provide a good variety of nutrients and also to reduce the risk of ingesting excessive nitrates. It would be of interest to study on the proportion of nutrients

and the appropriate amount of nitrate in solution of hydroponic fertilizer to minimize nitrate accumulation of green leafy vegetables growing under hydroponic method. Also since nitrate can be converted to nitrite in human body by the action of salivary bacteria, further investigation is needed to determine the risk of carcinogenic nitrosamine/nitrosamide formation in the gastrointestinal tract.

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ปริมาณไนเตรทของผักใบเขียวบางชนิดที่ปลูกด้วยวิธีที่แตกต่างกันในประเทศไทย

ชัชฎา เพชรเปี่ยมศิริ[*] ทิพวรรณ ศิริเตียรทอง[*] แก้ว กังสดาลย่ำไพ[*] ลินนา ทองยงค์[*]

บทคัดย่อ

อาหารที่มีไนเตรทสูงเพิ่มความเสี่ยงของโรคมะเร็งและโรคต่าง ๆ และผักเป็นแหล่งที่มาหลักของการได้รับไนเตรทเข้าสู่ร่างกาย การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาปริมาณไนเตรทของผักใบเขียว 4 ชนิด คือ ผักคะน้า ผักกวางตุ้ง ผักกาดขาว และผักกวางตุ้งฮ่องเต้ ที่ปลูกด้วยวิธีการปลูกที่แตกต่างกัน (วิธีดั้งเดิม, วิธีอนามัย, วิธีอินทรีย์ และวิธีไฮโดรโปนิกส์) ด้วยวิธีวิเคราะห์บรูซีน ในช่วงเดือนพฤศจิกายน พ.ศ. 2560 ถึงเดือนกุมภาพันธ์ พ.ศ. 2561 พบว่า ผักคะน้า ผักกวางตุ้ง และผักกาดขาวที่ปลูกด้วยวิธีไฮโดรโปนิกส์มีปริมาณไนเตรทสูงกว่าผักเหล่านี้ที่ปลูกด้วยวิธีอื่นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ผักกวางตุ้งฮ่องเต้ที่ปลูกด้วยวิธีดั้งเดิมมีปริมาณไนเตรทสูงกว่าผักกวางตุ้งฮ่องเต้ที่ปลูกด้วยวิธีอื่นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ผักกวางตุ้งไฮโดรโปนิกส์ (3,627 ± 900 มก./กก.) และผักกวางตุ้งฮ่องเต้ที่ปลูกด้วยวิธีดั้งเดิม (3,537 ± 817 มก./กก.) มีค่าไนเตรทสูงเกินกว่ามาตรฐานของสหภาพยุโรป (≤ 3,500 มก./กก.) เมื่อเปรียบเทียบวิธีการปลูก 4 วิธี พบว่า ผักที่ปลูกด้วยวิธีไฮโดรโปนิกส์มีค่าเฉลี่ยปริมาณไนเตรทสูงสุด (2,994 ± 958 มก./กก.) อย่างมีนัยสำคัญทางสถิติ แต่เมื่อเปรียบเทียบระหว่างผักทั้ง 4 ชนิด พบว่า ผักกวางตุ้งฮ่องเต้มีค่าเฉลี่ยปริมาณไนเตรทสูงสุด (2,634 ± 1,071 มก./กก.) อย่างมีนัยสำคัญทางสถิติ เนื่องจากทั้งวิธีการปลูกและชนิดของผักส่งผลกระทบต่อการสะสมไนเตรทในผักใบเขียว ดังนั้นการบริโภคผักที่หลากหลายชนิดในแต่ละมื้ออาจช่วยลดความเสี่ยงที่มนุษย์จะได้รับสารไนเตรทในปริมาณที่มากเกินไป

คำสำคัญ: การสะสมของไนเตรท, ผักใบเขียว, อินทรีย์, อนามัย, ไฮโดรโปนิกส์, ดั้งเดิม

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[*] ภาควิชาอาหารและเภสัชเคมี คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย