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Determination of Nitrate in the Edible Part of Vegetables from Markets Around Chiang Mai City, Northern Thailand by using High Performance Liquid Chromatography

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ABSTRACT

Vegetables play a popular food among consumer who needs the healthy food in Chiang Mai, Thailand. The quality and safety food was the main thing that is guarantee for vegetable products which sold in the markets. Nitrate is the one chemical that has reported contamination in vegetables. The level of nitrate content in vegetable products from hydroponic and conventional system will be interested. So, this study aimed to investigate the amounts of nitrate in vegetable products from hydroponic and conventional vegetable products which sold in markets around Chiang Mai city. The common vegetables were 3 cross-sectional sampling from retailer market in 2013 around Chiang Mai city, northern Thailand. All samples were prepared and measured the nitrate content using HPLC method. The mean nitrate concentrations in vegetable products from hydroponics and conventional system were compared. There was found that nitrate concentrations in lettuce hydroponics products were significantly higher than conventional in every season. Kale, flowering Chinese cabbage and Pak Choi contained very high nitrate contents with mean of nitrate concentrations higher than 3,000 mg kg^{-1} (fresh weight). In conclusion, the level of nitrate in both hydroponic and conventional lettuces samples from Chiang Mai city, northern Thailand in 2013 were lower than EU-MRL except conventional kale, flowering Chinese cabbage and Pak Choi samples have the high concentration but no MRL established yet. In comparison of type of markets show no significant difference of nitrate content in vegetable products so consumer can be bought less nitrate contamination in vegetable from all type of markets.

Key words: Nitrate content, Chiang Mai, northern Thailand

INTRODUCTION

Consumers are increasingly interested in eating diets rich in fruits and vegetables because of the purported health benefits (Martin *et al*., 2012; Dickson-Spillmann and Siegrist, 2010; Efstratiou, 2009). Nitrate is a form of nitrogen that plants need for growth (Jan and Ahmed, 2013). Large amounts of nitrate may be present in leafy vegetables. In various vegetables, the amount of nitrate depends on factors such as cultivation processes, variety of plant, plant age, growing season and types of nitrogen fertilizer (Ali *et al*., 2013). The ratio of nitrate reduction in root rises by increasing the environment temperature. However, excessive fertilizing in each phase leads to

increasing nitrate concentration in the plant (Abayomi *et al.*, 1988). Nitrate can be changed to nitrite by the enzyme, nitrate reductase, with molybdenum as cofactors (Haq *et al.*, 1999). Then, nitrite can also convert to ammonia which is included as a component of organic glutamine. Nitrite can react with amines in food and become to nitrosamine which is a carcinogen. The nitrosamines have caused cancer in liver, stomach and esophagus. On the other side, the positive effects of nitrite were reported that can be regulated of blood pressure (Bahra *et al.*, 2012; Kapil *et al.*, 2010). Although, the effect of nitrate is good or bad or the amount is more or less the underlying data is an important factor in the education of the future.

Chiang Mai province is located on northern part of Thailand where it is one of the large cities with many famous tourist attractions and business centers and a lot of people living for working and traveling. Because of increasing consumption of healthy food (Martin *et al.*, 2012), vegetable salad becomes a popular dish and the demand of high-quality and residue-free vegetable products especially lettuce (*Lactuca sativa* L.) are increasing. The content of contaminated compounds which can lead to health adverse effects in vegetable products is becoming a vital consideration for vegetable producers and growers (Evans *et al.*, 2012; Dickson-Spillmann and Siegrist, 2010). However, there is no data about nitrate content in leafy vegetable available in Chiang Mai, Thailand.

Therefore, the goal of present study was to determine the nitrate content in fresh vegetables which are sold in markets (supermarket, super store and wet-markets) with difference crop cultivation system, i.e., hydroponics and conventional cultivation in Chiang Mai province, Thailand.

MATERIALS AND METHODS

Determination of nitrate in vegetable samples

Samples collection: The present study was 3 cross-sectional study, fresh vegetable were collected in 3 seasons (summer, winter and rainy) of 2013 from supermarkets, superstores and wet market around Chiang Mai city, Thailand. Nine types of vegetables, which are the most commonly consumed vegetables during the whole year, were brought from markets. All vegetable samples labeled the information about cultivation systems as conventional, organic and hydroponic were purchased from retailer market around Chiang Mai city in January, April to June and August 2013. The sampling vegetables were the lettuce (*Lactuca sativa* var. crispa L.) product consisted of lose leaf lettuces (green leaf lettuce, red leaf lettuce, green oak, red oak), Cos lettuce or Romaine Lettuce (*Lactuce sativa* longifolia), Butter head (*Lactuca sativa* L.) and the others were Kale (*Brassica oleracea* var. alboglabra), flowering Chinese cabbage (*Brassica camprestris* L. chinensis (Lour.) Rupr.) and Pak Choi (Brassica Chinensis var. Chinensis Mansf.). After purchasing, the samples were transported to the Toxicology laboratory, Environment and Health research unit, Research Institute for Health Science, Chiang Mai University, Chiang Mai, Northern Thailand.

Preparation of samples: The inedible portions of vegetable samples were removed and washed with tap water followed by distilled water, then dabed by tissue paper and air dried. The samples were chopped and homogenized using homogenizer (IKA-T25 digital Ultra-Turrax, Germany) for 30 sec and immediately stored at -20°C until analysis.

Sample extraction: The method of extraction was modified from Chou and colleagues' method (Chou *et al.*, 2003). In brief, each sample was finely chopped and homogenized. One gram of

homogenized vegetable was weighed in 125 mL Erlenmeyer flask and added with 50 mL of type I water. The flask was incubated in a shaking water bath for 20 min, at 80°C. After cooling down, the supernatant was adjusted to 100 mL final volume with type I water and filtered through a 0.45 μm filter.

Quantification of nitrate content in vegetable samples by HPLC analysis: The nitrate content in all samples was quantified by HPLC. Ten microliters of the sample were injected into HPLC system (Agilant 1100) equipped with auto-sampler. Analyses were performed at 40°C and UV/Vis-detector was set at 213 nm. Nitrate was eluted from VertiSep GES C18 HPLC column (250×4.6 mm, 5 μm; Vertical, Thailand) with 20% methanol (pH 7.0) with 0.01 M octylammonium orthophosphate at a flow rate of 0.8 mL min^{-1}. The method efficient parameters i.e. precision, accuracy, Limit of Detection (LOD) and Limit of Quantization (LOQ) were derived from pooled vegetable mixing.

Statistical analysis: The results of nitrate content were express as mean of mg kg^{-1} fresh weigh (f.w.)±Standard Deviation (SD). The differences between the mean values were performed by using ANOVA test. All tests were performed at the 0.05 level of significance.

RESULTS

Method validation of the nitrate content in samples was performed by using $NaNO_3$ standard curve. Method validations for nitrate determination were carried out using pooled vegetable samples. The linearity was 0.999 with working range between 0.1-100 μg mL^{-1}. LOD and LOQ, defined as 3 and 10 times signal-to-noise ratio, were 0.01 and 0.1 mg kg^{-1}, respectively. The accuracy and precision were reported by percentages of recovery by using pooled samples. The recovery of spiked 1.0, 10.0 and 100 μg mL^{-1} $NaNO_3$ samples were 94.9±2.3, 111.7±6.2 and 103.7±12.4, respectively. The intra-batch and inter-batch coefficients of variation were 2.01% (n = 10) and 2.10% (n = 31), respectively. The validation results were shown in Table 1.

The mean nitrate levels in all lettuce samples were show in Table 2. The hydroponic lettuce products in all seasons had mean nitrate content range 2,604-2,809 mg kg^{-1}

Table 1: Method performance data for nitrate analyzing in pooled vegetable samples by HPLC-UV

Analytical parameters	Performance data
Linearity	0.999
LOD	0.01
LOQ	0.10
Recovery (%)	94.9-111.7
Intra-batch (%), n = 10	2.01
Inter-batch (%), n = 31	2.10

Table 2: Nitrate content in lettuces samples (mg kg^{-1} f.w.) from hydroponic and conventional fresh vegetable products sold in variant markets in 3 seasons of 2013

	Hydroponic products			Conventional products		
Seasons	N	Range	Mean±SD	N	Range	Mean±SD
Winter	29	1,416-4,640	2,809±616	41	141-4,640	1,332±817
Summer	20	1,593-3,517	2,604±507	43	83-2,875	1,176±633
Rain	33	756-3,100	2,759±833	46	50-3,100	1,440±715

Table 3: Nitrate content in Pak Choi, flowering Chinese cabbage and kale (mg kg^{-1} f.w.) from conventional fresh vegetable products sold in variant markets in 2013

	Super markets			Super stores			Wet-markets		
Vegetables	N	Range	Mean±SD	N	Range	Mean±SD	N	Range	Mean±SD
Pak Choi flowering	12	1,163-4,957	2,994±1,263	14	1,381-6,516	3,728±1,616	18	619-5,232	2,961±1,572
Chinese cabbage	10	589-5,005	2,818±1,360	28	1441-5,199	3,686±975	22	290-6,430	2,945±1,708
Kale	13	870-5,592	2,700±1,450	7	801-5,556	3,374±1,470	28	291-5,136	2,554±1,523

fresh weight (756-4,640 mg kg^{-1} fresh weight). The conventional lettuce products had mean nitrate content range 1,176-1,332 mg kg^{-1} fresh weight (50-4,640 mg kg^{-1} fresh weight).

Pak choi, kale and flowering Chinese cabbage are highly-demanded vegetables in Chiang Mai province and all other around these area. Because of small number of the hydroponic products that may have difficulty to produce but the samples of hydroponic product were analyzed and had very high concentration. So, the present study shown only results of nitrate content in Pak Choi, kale and flowering Chinese cabbage from conventional products which sold in variant markets. The mean of nitrate content in all vegetable had no significant difference between 3 types of markets were in Table 3. These three vegetables from superstore were shown the highest nitrate content while the nitrate concentration of products from wet-markets had very board range.

DISCUSSION

According to the method showed good validity which has good precision, high accuracy and sensitivity. The result of present study showed nitrate in hydroponics products higher than conventional products. Some previous studies reported the difference between the nitrate levels in vegetables harvested in winter and summer (Gent, 2011; Escobar-Gutierrez *et al*., 2002). However, our results demonstrated the opposite findings. This disparity in data may be due to the different environmental conditions (i.e. length of exposure to sunlight, cultivation methods and seeding time). The lettuce samples in this study had lower nitrate contents than EU standard which limits nitrate contents in lettuces grown under cover and open air at 5,000 and 4,000 mg kg^{-1} fresh weigh, respectively.

The nitrate content in lettuce products had no significant difference between winter, summer and rainy season seasons within the same type of cultivation. The hydroponic product had significantly higher nitrate than conventional product with significant difference ($p<0.05$) (Fig. 1).

Determination of nitrate content in lettuces sample in variant markets were shown in Fig. 2, the hydroponic lettuces products sold in the markets were had higher concentration of nitrate than conventional product with significant difference ($p<0.05$) but no significant in different kind of markets. Among hydroponic products, samples from superstore had the highest nitrate contents while the highest nitrate-containing conventional products were from wet market. The ratio of nitrate concentration between hydroponic and conventional products sold in supermarkets, superstores and wet-markets were 2.18, 2.37 and 1.76, respectively.

The nitrate contents in all kinds of samples from conventional production were shown in Fig. 3. The lettuce product had low nitrate than others with significant difference ($p<0.05$). Pak Choi, flowering Chinese cabbage and kale were not difference in nitrate concentration among all seasons.

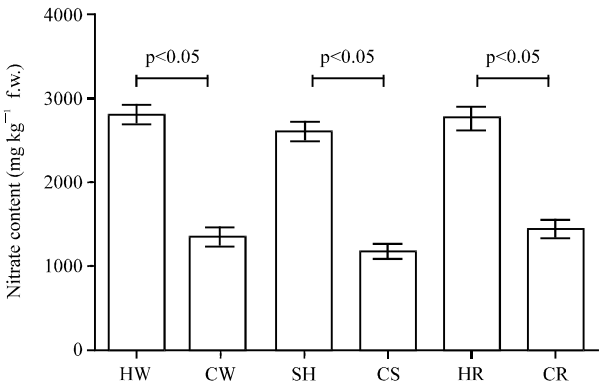

Fig. 1: Nitrate content in hydroponic and conventional lettuces product in difference seasons of 2013. HW: Hydroponic in winter, CW: Conventional in winter, HS: Hydroponic in summer, HR: Hydroponic rainy, CR: Conventional rainy

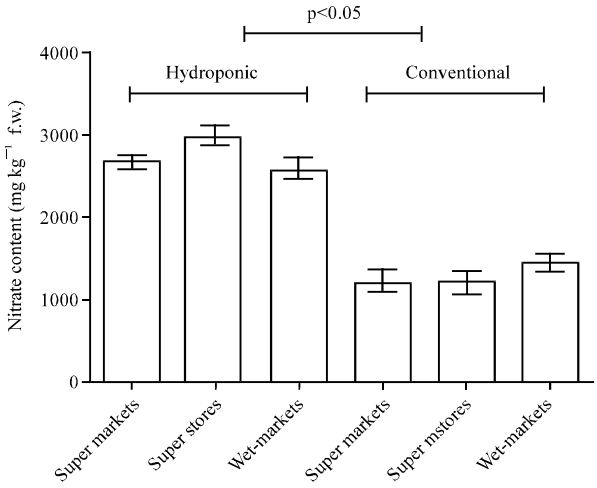

Fig. 2: Nitrate content in lettuces hydroponic and conventional products from supermarkets, superstores and wet-markets around Chiang Mai City, Thailand in 2013

Kale, flowering Chinese cabbage and Pak Choi which are popular leafy vegetables among Thai consumers contained very high nitrate contents. There were Pak Choi, flowering Chinese cabbage and kale sold in superstores had mean of nitrate concentrations higher than 3,000 mg kg^{-1} fresh weight, this level has to be concerned and aware when consuming these kinds of vegetables especially fresh products. The level of nitrate in lettuce from both hydroponic and conventional products had lower than EU standard (EU, 2011). The results showed those nitrates content in vegetables which were significantly affected by the system of cultivation. The vegetable products from hydroponic system had highest nitrate content. However, the nitrate accumulation in vegetables may depend on plant size, harvesting period, nitrate level and form which were applied to vegetable and other environmental factors (Tosun and Ustun, 2004; Zhou *et al*., 2007; Gent, 2011). The result from present study was similar to the previous study which reported the level of nitrate in same kinds of vegetables.

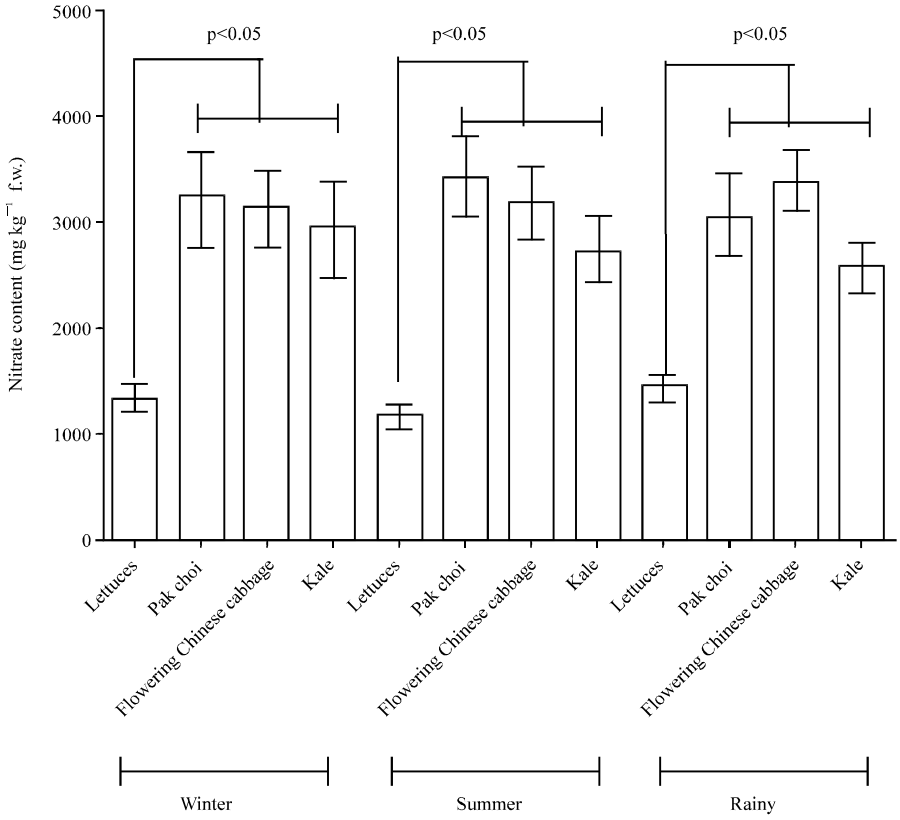

Fig. 3: Nitrate content in fresh vegetable from conventional production from difference seasons

CONCLUSION

We have demonstrated the presence of high concentration of nitrate in hydroponic lettuce products which was well-known as pesticide-free product and healthy diet. The level of nitrate in vegetable samples from Chiang Mai city, northern Thailand in 2013 was lower than EU-MRL except flowering Chinese cabbage and Pak Choi in vegetables product. Although, some studies reported vegetables play a good source for antioxidant, help to decrease hypertensions and other beneficial agents who act as anti-cancer and health promotion, consumers should consider about the harmful of the nitrate content in vegetables. It is necessary to establish limits of nitrate contents in vegetable in Thailand.

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