Nitrate and Nitrite Contents of Vegetables Marketed in Chiang Mai Province

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ABSTRACT

Determination of nitrate and nitrite contents of 33 kinds of vegetables marketed in Chiang Mai province and 4 kinds of self-planted vegetables without using chemical fertilizer was carried out. It was found that in marketed vegetables, 18 kinds had high nitrate contents, 15 kinds had low nitrate contents and 3 out of 4 kinds of self-planted vegetables had high nitrate contents comparable to marketed vegetables. The reason that nitrate contents were not different might be due to potassium nitrate fertilizer that was used for nourishing leafy vegetables and the fertilizers that persist in soil. The low nitrate contents in marketed vegetables might result from the fact that vegetables had changed them into protein and uric acid. Eating the vegetables for the adults is safe because some vegetables contain vitamin C and they also take 5 types of principle foods. Vitamin C inhibits the chemical reactions between nitrite and amino compound that otherwise becomes N-nitroso compound which is carcinogenic.

The results from this investigation suggest that we must be careful in using boiled vegetable soup as supplemental food for babies under 3 months old. These babies do not have NADH-methemoglobin reductase which is used for changing methemoglobin-Fe^{+3} to hemoglobin-Fe^{+2}, and the new-born babies have hemoglobin-F 60-80% which is oxidized by nitrite into methemoglobin more easily than hemoglobin A. These reactions may cause the babies' death.

Key words: Nitrate, Nitrite, Vegetables

INTRODUCTION

Nitrate and nitrite are always found as intended or nonintended presevatives in food. Nitrate salt is a component of inorganic fertilizer potassium nitrate. In agricultural areas which this fertilizer is used, we will find nitrate in soil, water, plants and meat. Nitrate is the ferltilizer that nourishes leaves so we will find nitrate in vegetables. We may also receive nitrate indirectly from drinking water, tap-water, milk, fruits and meat. Food producers use nitrate and nitrite in preserved meat such as sausage, ham and canned meat and also in pickled vegetables. Sodium or potassium nitrate and nitrite can prevent the growth of *Clostridium botulinum*. Nitrate

and nitrite can digest meat and it also becomes red and fresh because nitrate and nitrite will combine with heme or hemoglobin and give the red substance. From experimentation, we found that food producers should add about 20-50 mg of nitrite in 1 kg meat which would produce red meat and good taste. (Suttichit, 1988)

Natural contents of nitrite in water, soil and plants are low but it may come from nitrate by biological change or reduction reaction by denitrifying bacteria. Nitrite will react with amino compound and becomes nitrosamine or N-nitroso compound which is carcinogenic. Vitamin C in fruits and many kinds of vegetables can inhibit this reaction as following : (Suttichit, 1988)

$$NaNO_3 + NADPH + H^+ \xrightarrow[\text{in bacteria}]{\text{Nitrate reductase}} NaNO_2 + NADP^+ + H_2O$$

$$NaNO_2 + HCl \rightleftharpoons HNO_2 + NaCl$$

$$2NHO_2 \rightleftharpoons N_2O_3 + H_2O$$

$$(CH_3)_2NH + N_2O_3 + e^- \xrightleftharpoons{\text{Vit C}} (CH_3)_2N{-}N{=}O + NO_2^-$$

N-Nitrosodimethylamine

The maximum limit of nitrate in preserved meat such as ham, sausage etc. is 500 mg/kg (calculated as sodium nitrate content) and the limit in mineral water and drinking water in closed bottle one 50 mg/L and 4 mg/L respectively (calculated as nitrogen content). The maximum limit of nitrite in preserved and canning meat are 125 and 50 mg/kg respectively (calculated as sodium nitrite content) and the limit in mineral water is 20 mg/L. There is no safety limit in vegetables. (Thai Food Act 2004). The present investigation reports on the amounts of nitrate and nitrite in marketed vegetables whether they are safe or not.

MATERIALS AND METHODS

Sample materials and preparation of samples

The sample materials consisted of 33 kinds of vegetables bought from 3 Chiang Mai market places in April 2005 plus 4 kinds of chemical fertilizer free, self-planted vegetables in Amphur Hang Dong, Chiang Mai province at the same time.

Ten grams of homogenized plant sample were mixed with 70 ml distilled water and pH adjusted to 8.0 with 12 ml of 2% sodium hydroxide solution. The mixture solution was then transferred to a 200 ml volumetric flask and warmed to 50-60°C in a water bath. Ten milliliters of 0.42 M zinc sulfate were added into the sample mixture and kept continuous warmed for further 10 minutes. Swirling and cooling the sample to room temperature before adjusting the volume to 200 ml with distilled water and followed by filtration.

Determination of nitrite contents

Ten milliliters of sample was pipetted into 50 ml volumetric flask. Ammonium chloride buffer 9.0 ml, acetic acid 60% 5.0 ml and color reagent 10.0 ml (mixture of sulfanilic acid solution 1% and N-(1-naphthyl)-ethylenediamine dihydrochloride solution 0.1% in equal volume) were added into the sample filtrate and diluted to 50 ml with distilled water. The sample mixture stored in a dark place for 25 minutes and the absorbance was measured at 550 nm. The nitrite contents were calculated from calibration curve. (Sen and Donaldson, 1978)

Determination of nitrate contents

The cadmium column which had been tested for the efficiency was washed with 25 ml ammonium chloride buffer. The sample filtrate was diluted with distilled water in order to have optimum amount of nitrate which cadmium column could reduce to be nitrite completely. This adjusted sample 10.0 ml was mixed with ammonium chloride buffer 5.0 ml and this sample mixture was poured through cadmium column. The column was washed with 15.0 ml distilled water and the total solution was transferred into 50 ml volumetric flask. Sixty percent acetic acid 5.0 ml and the color reagent 10.0 ml were added into the sample solution and it was diluted to 50.0 ml distilled water. The sample was shaken and stored in a dark place for 25 minutes. The absorbance was measured at 550 nm, comparing with reagent blank which was prepared by using the same method as the sample but 10.0 ml distilled water was used instead of sample solution. (Sen and Donaldson, 1978)

RESULTS

Nitrate and nitrite contents of 33 kinds of vegetable, marketed in Chiang Mai province and 4 kinds of self-planted vegetable without using chemical fertilizer were examined. It was found that in marketed vegetables, 18 kinds had high nitrate contents, 15 kinds had low nitrate contents and 3 out of 4 kinds of self-planted vegetables had high nitrate contents similar to makerted vegetables. (Tables 1,2 and 3)

The maximum limit of nitrate in preserved meat is 500 mg/kg. (Calculated as sodium nitrate content) and the limit in mineral water and drinking water in closed bottle are 50 mg/L and 4 mg/L (calculated as nitrogen content). The maximum limit of nitrite in preserved and canning meat are 125 and 50 mg/kg (Calculated as sodium nitrite content) and the limit in mineral water is 20 mg/L. (Thai Food Act 2004). There is no safety limit in vegetables.

Table 1. Vegetables with high amounts of nitrate and nitrite bought from Chiang Mai markets in April 2005.

No	Vegetable Types	Nitrate amounts* (mg/kg)			*Nitrite amounts (mg/kg)		
		Market places			Market places		
		1st	2nd	3rd	1st	2nd	3rd
1	Phak kana (*Brassica alboglabra*)	5195	5677	6119	19	15	-
2	Khuen chai (*Apium graveolens*)	2194	5167	4633	-	-	-
3	Phak poileng	1732	1650	4233	-	-	-
4	Phak kwang tung	2873	2145	3133	-	-	-
5	Chai thao (*Raphanus sativus*)	3023	2182	744	-	-	-
6	Phak chi (*Coriandrum sativum*)	997	2358	2653	6	2	2
7	Kui chai (*Allium tuberosum*)	2534	1126	1000	-	16	-
8	Phakkat khao (*Brassica laxa*)	2170	2356	254	-	-	-
9	Phak tang O (*Chrysanthemum coronatum*)	540	627	1958	-	-	-
10	Phakkat hom (*Lactuca sativa*)	1597	690	1110	-	-	-
11	Ton hom	1391	1390	914	-	-	-
12	Phak bung Cheen (*Inula sp.*)	1286	677	731	-	-	-
13	Phak hung hong (*Hedychium coronarium*)	224	127	1153	-	-	-
14	Phak salat kaeo (*Lactuca sp.*)	872	449	805	-	-	-
15	Saranae (*Meutha codifolia*)	829	117	186	-	-	-
16	Broccoli (*Brassica oleracea Var. botrytis)*	823	280	329	-	-	-
17	Kalam dok (*Brassica oleracea*)	760	785	27	-	-	-
18	Mara (*Momordica charantia*)	361	110	375	-	-	-

* Average from duplicated sample

- Undetectable

Table 2. The low amounts of nitrate and nitrite in vegetables bought from Chiang Mai markets in April 2005.

No	Vegetable Types	Nitrate amounts* (mg/kg)			*Nitrite amounts (mg/kg)		
		Market places			Market places		
		1st	2nd	3rd	1st	2nd	3rd
1	Phak kana (*Brassica alboglabra*)	232	427	327	-	-	-
2	Horapha (*Ocimum bacilicum*)	320	273	183	-	-	-
3	Thua fak yao (*Vigna unguiculata*)	100	195	78	-	-	-
4	Phak plang (*Basella rubra*)	130	59	175	-	-	-
5	Phak wan (*Phyllanthus geoffrayi*)	93	168	102	-	-	-
6	Kalam pli (*Brassica capitator*)	13	12	54	-	-	-
7	Buap (*Luffa acutangula*)	46	20	15	-	-	-
8	Phrik wan (*Capsicum sp.*)	23	8	24	-	-	-
9	Phrik chifa (*Capsicum acuminatum*)	10	20	7	-	-	-
10	Phrik yuake (*Capsicum annuum*)	7	5	17	-	-	-
11	Thua lan tao (Pisum sativum)	12	12	7	-	-	-
12	Phak kachet (*Neptunia oleracea*)	11	7	4	-	-	-
13	Phrik khinu (*Capsicum frutescens*)	10	9	7	-	-	-
14	Thua ngork (*Vigna radiata*)	10	4	5	-	-	-
15	Cha om (*Acacia pennat subsp. insuavis*)	3	-	2	-	-	-

*Average from duplicated samples

- Undetectable

Table 3. Amounts of nitrate and nitrite in self-planted vegetables in Amphur Hang Dong, Chiang Mai province with out using chemical fertilizer in April 2005.

No.	Vegetable Types	Nitrate amounts* (mg/kg)	Nitrite amounts* (mg/kg)
1	Phakkat khao (Brassica pekinensis var. laxa)	2239	3
2	Phak kwang tung	1854	-
3	Phakkat khiao (Brassica juncea)	1207	-
4	Phak bung (Inula aquatica)	294	-

*Average from two time determinations

- Undetectable

DISCUSSION AND CONCLUSION

The high nitrate contents in marketed and self-planted vegetables may come from intended potassium nitrate and the fertilizers that persisted in the soil because we do this experiment in summer. Potassium nitrate is the chemical fertilizer that nourishes leaves so Phak kana and Phakkat Khao which we eat their leaves had high

nitrate contents but Kalam dok which we eat its flowers had low nitrate contents. (see Table 1) Phakkat Khao is the vegetable that we make it as boiled vegetable soup and use it as supplemental food for babies under 3 months old so it is dangerous because the babies do not have NADH-methemoglobin reductase which is used for changing methemoglobin-Fe^{+3} into hemoglobin-Fe^{+2}. The new-born babies have hemoglobin-F 60-80% which is oxidized by nitrite into methaemoglobin more easily than haemoglobin A so eating boiled vegetable soup that contains high nitrate amount may cause the babies' death. (Suttichit, 1988).

Nitrate in vegetables can change to be protein and uric acid so these vegetables had low nitrate contents such as Cha om, Phak krachet, Thua lan tao and Thua fak yao had low nitrate contents.

Thua ngork had nitrate content only 4 mg/kg. The nitrate content was low because Thua ngork usually grown in water which has low nitrate content. (see Table 2)

Vegetables that grow in winter had high nitrate content more than that grow in summer. (Food Standard Agency, 2001).

Phrink wan, Phrik chifa, Phrik yuake and Phrik khinu have oleoresin as the major component because they suck this component from the soil so they have low nitrate content.

Thai maximum limit of nitrate in meat is 500 mg/kg so it is in the same ADI limit in vegetable of UK. (Thai Food Act 2004, Food Standard Agency, 2001)

The highest amount of nitrate was found in Phak kana which was 6119 mg/kg. When we eat these vegetables, we are still safe because some vegetables contain vitamin C and we also take 5 types of principle foods, so we still receive vitamin C. This vitamin can inhibit nitrite and amino compound from changing into N-nitroso compound which is carcinogenic. (Suttichit, 1988)

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