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Thiamin content and activity of antithiamin factor in vegetables of southern Thailand

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Fourteen types of vegetable commonly consumed by people in southern Thailand were analyzed for content of endogenous free-form thiamin and activity of antithiamin factor (ATF). The mean of endogenous free-form thiamin content (μg/g vegetable) ranged from 0 in phak kuad (*Diplazium esculentum* (Retz.) SW.) and khanun (*Artocarpus heterophyllus* Lamk.) to 2·8 in sato (*Parkia speciosa* Hassk.). Activity of heat-stable ATF incubated for 3 h (percent thiamin destroyed/g vegetable) ranged from 2·3 in sato to 100 in phak kuad and khanun. Among the analyzed samples, activity of heat-stable ATF above 50% was found in 10 types. Activity of heat-labile ATF above 40% was found only in four types and the rest were below 15·7%. Furthermore, samples with a high activity of ATF tended to have low thiamin content.

INTRODUCTION

Inadequate thiamin intake or consumption of antithiamin factors can result in thiamin deficiency. Antithiamin factors can be divided into two types: (1) compounds similar in structure to thiamin or thiamin analogues which compete with thiamin to bind with apoenzyme and interfere with haloenzyme function; and (2) the structure-modifying antithiamin compounds, including enzymatic heat-labile compounds such as thiaminase I, and heat-stable antithiamin factors such as tannic acid in plants. Thiamin deficiency is still a nutritional problem in various areas of Thailand, a country where rice is the staple food. It has been reported that vegetables locally grown and consumed in north and northeastern Thailand contain heat-stable antithiamin factors. Betel nut (*Areca catechu* Linn.) is an example of a vegetable containing antithiamin factor activity (Nakornchai *et al.*, 1975). The consumption of these vegetables has been demonstrated as a factor leading to poor thiamin status among the population. The assessment of thiamin status was based on the measurement of erythrocyte transketolase activity and its percent stimulation resulting from the addition of thiamin pyrophosphate (TPP). Vimokesant *et al.* (1974) showed that the withdrawal of fermented tea leaves from the diet of northern people decreased percent TPP stimulation from 20·6 ± 3·3 to 14·9 ± 2·6, while chewing of fermented tea leaves together with supplementation of thiamin showed no decrease in percent TPP stimulation. The withdrawal of betel nut plus cooking of fermented fish (to destroy thiaminase) significantly decreased percent TPP stimulation from 16·4 ± 1·5 to 6·6 ± 1·1 in northeastern Thai subjects, while resumption of betel nut chewing showed an increase in percent TPP stimulation to 12·7 ± 2·0 (Vimokesant *et al.*, 1975). Results of these studies suggested that frequent consumption of food containing high antithiamin factors, such as fermented tea leaves and betel nut, leads to lower levels of thiamin than normal, although the consumption of thiamin is adequate among the population. In southern Thailand, people have a habit of eating rice as a staple food with several kinds of raw and fresh vegetables, and fermented fish (a diet similar to that consumed by people in the northern and northeastern parts of Thailand). Sato (*Parkia speciosa* Hassk.), rieng (*Parkia timoriana* (DC.) Merr.), nieng (*Archidendron jiringa* (Jack) Nielson) and mamuang himmaphaan (*Anacardium occidentale* Linn.) are examples of vegetables mainly grown along the southern peninsula and typically consumed in the southern area, which have not yet been analyzed for their content of antithiamin factors. This study is aimed at determining whether vegetables locally grown and consumed in the southern area possess antithiamin factors. Concurrently, the content of endogenous free-form thiamin is also determined.

MATERIALS AND METHODS

Three lots of fresh vegetables were used for determination of content of endogenous free-form thiamin and activity of antithiamin factors at 60°C and 37°C according to the following procedures.

Preparation of vegetable extracts

In each analysis fresh vegetables were bought from local markets in Hat Yai city and brought to the laboratory. They were then cleaned and the edible parts quartered into 25-g samples. The 25-g sub-sample of each vegetable was finely blended in a Waring Blender (2-Speed, 3390-D66, USA) at maximum speed for 10 min in 100·0 ml of 0·1 M phosphate buffer solution, pH 7·5. The homogenate was further filtered through thin cloth and centrifuged at 3000 rpm (1000 *g*) for 10 min in a bench top centrifuge (Damon/IEC HN-S II, USA). The supernatant was collected and labeled as a crude extract of vegetable. Each 2·0 ml of this crude extract was equivalent to 0·5 g of vegetable mass. The freshly prepared crude extracts were then used to determine endogenous free-form thiamin content and activity of antithiamin factors according to the methods described below.

Determination of thiamin

In each analysis the amount of endogenous free-form thiamin in freshly prepared crude extract of vegetables was determined in duplicate by the method of György and Pearson (1967). The assay mixture containing a crude extract of vegetable (2·0 ml) was mixed with 0·02 M citrate buffer, pH 5·5 (2·0 ml) and made up to the final volume of 10·0 ml with water. The assay mixture was adjusted to a pH of 3·5, loaded onto a Decalso (Permutit T, Fisher Scientific Company, USA) column and eluted with 25% KCl–0·1 N HCl solution. Eluted thiamin was further oxidized to thiochrome with 1% $K_3Fe(CN)_6$:15% KOH (1:10 by volume ratio). The fluorescent intensity of thiochrome extracted with isobutanol was determined in a spectrofluorometer at an excitation wavelength of 375 nm and an emission wavelength of 425 nm. The amount of thiamin was read from a standard curve which was constructed similarly to the sample by replacing the crude extract of vegetable with standard thiamin in amounts of 1, 2 and 5 μg. Using this procedure, adjustment of the obtained values by percentage recovery of thiamin from the Decalso column is not required (average recovery of standard thiamin from the column was 95% within the range of 0–5 μg).

Using infant formula milk powder for quality control, duplicate samples simultaneously carried through the assay procedure gave values agreeing within 2·7% of the mean, while assays of homogeneous material made on different days gave values agreeing within 5·5% of the mean.

Determination of activity of antithiamin factors

In each analysis, activity of antithiamin factors was determined in duplicate using freshly prepared crude extract of vegetables. The assay mixture was prepared by adding standard solution of thiamin (10 μg) to a crude extract of vegetable (2·0 ml) and adjusting to the final volume of 10·0 ml with 0·1 M phosphate buffer, pH 7·5. The mixture was then incubated at 60°C for 3 h. After incubation, 2·0 ml of the mixture was used to determine the amount of free thiamin remaining, as described above. For the determination of activity of heat-labile antithiamin factor, the same procedure was followed except that the temperature was maintained at 37°C. The activity of antithiamin factors was expressed as percent destruction of added thiamin/g vegetable after 3 h incubation by the following formula:

$$\%\ \text{thiamin destroyed/g vegetable} = \frac{(B_A + B_S - B_L) \times 100}{(B_A + B_S) \times 0{\cdot}5}$$

where B_A is the amount of free thiamin added before incubation in μg units, B_S is the amount of endogenous free-form thiamin in a crude extract of vegetable in μg units, B_L is the amount of free thiamin left after incubation in μg units, and 0·5 is the weight in grams of fresh vegetable equivalent to 2·0 ml of the crude extract.

RESULTS AND DISCUSSION

The local name, common name, a description of the part used for the analysis, and the scientific name of 14 vegetables commonly consumed by people in southern Thailand are shown in Table 1. As shown in Table 2 sato (*Parkia speciosa* Hassk.) contained the highest amount of endogenous free-form thiamin (2·8 μg/g vegetable) followed by germinating nieng (1·5 μg/g vegetable). Khanun and phak kuad contained only trace amounts of thiamin, 0·0 μg/g vegetable.

Results of percentage destruction of added thiamin indicated that the activity of antithiamin factors (ATF) was detected in all of the assayed vegetables and, in most, the ATF was much higher at 60°C than at 37°C (Table 2), similar to results of Nakornchai *et al.* (1975). This finding indicates that assayed vegetables had a higher content of heat-stable than of heat-labile antithiamin factor. At 60°C, ATF values of the assayed vegetables ranged from 2·3% in sato to 100% in phak kuad and khanun. At 37°C, their ATF values ranged from 0·0% in kra thin, cha muang, kheelek and ma kok to 100% in phak kuad and cha phluu.

A scatter plot of mean activity of antithiamin factor against mean content of endogenous free-form thiamin (Fig. 1) shows that vegetables with high activities of antithiamin factors, both heat-stable and heat-labile, had low levels of endogenous free-form thiamin. For example, a group of vegetables with activity of heat-stable antithiamin factor above 85%, such as phak kuad, khanun, phak waen, bua bok, cha phluu, kra thin and fak thong, had thiamin contents below 0·7 μg. A group of vegetables with activity of heat-labile antithiamin factor above 40%, such as phak waen, khanun, phak kuad and cha phluu, had thiamin contents in the range of 0–0·2 μg. The plot also shows clearly that vegetables

Table 1. Assayed vegetables commonly grown and consumed by people in southern Thailand

Local name	Abbreviation	Common name (parts used)	Scientific name[a]
Bua bok	B	Pennywort (leaves)	*Centella asiatica* Urban
Cha muang	Cm	Cha muang (leaves)	*Garcinia cowa* Roxb.
Cha phluu	Cp	Cha phluu (leaves)	*Piper sarmentosum* Roxb.
Fak thong	F	Pumpkin (leaves)	*Cucurbita moschata* Decne
Khanun	Kn	Jackfruit (fruits)	*Artocarpus heterophyllus* Lamk.
Kheelek	Kl	Kheelek (leaves)	*Cassia siamea* Lamb.
Kra thin	Kt	Lead tree (leaves)	*Leucaena leucocephala* (Lamk.) De Wit.
Ma kok	Mk	Hog plum (leaves)	*Spondias pinnata* Kurx.
Mamuang himmaphaan	Mh	Cashew nut (leaves)	*Anacardium occidentale* Linn.
Nieng	N	Nieng (seeds)	*Archidendron jiringa* (Jack) Nielson
Nieng (germinating)	N(g)	Germinating nieng (seeds & roots)	*Archidendron jiringa* (Jack) Nielson
Phak kuad	Pk	Phak kuad (leaves)	*Diplazium esculentum* (Retz.) SW.
Phak waen	Pw	Phak waen (leaves)	*Marsilea crenata* Presl.
Rieng	R	Rieng (seeds)	*Parkia timoriana* (DC.) Merr.
Sato	S	Sato (seeds)	*Parkia speciosa* Hassk.

[a] Data are from Nielson (1985), Smitinand (1980) and Tagawa and Iwatsuki (1988).

with highest endogenous free-form thiamin content such as sato (2·8 μg) had low activities of both heat-stable and heat-labile antithiamin factors.

Although few clinical surveys on thiamin status have been reported, there is evidence for the existence of thiamin deficiency in the southern population. Assessment of thiamin status in women of southern Thailand by Ongroongruang (1991) revealed that 5 out of 14 non-pregnant subjects (35·7%) and 8 out of 10 mothers (80%) have a TPP stimulation above 15% (poor thiamin status). Frequent consumption of polished rice, low consumption of energy and protein among the population, who spend most of their time on the rubber plantation or the rice field, or fishing, are possible causes leading to various nutritional problems, including poor thiamin status. Out of 292 infants born in the Hat

Table 2. Endogenous free-form thiamin content and activity of antithiamin factors in vegetables commonly grown and consumed by people in southern Thailand

Local name	Thiamin content[a] μg thiamin/ g vegetable	Activity of antithiamin factor[a] %thiamin destroyed/g vegetable	
		60°C	37°C
Phak kuad	0·0 (0·0)	100 (0·0)	100 (0·0)
Khanun	0·0 (0·0)	100 (0·0)	73·5 (0·0)
Phak waen	0·1 (0·0)	92·0 (8·3)	41·8 (29·3)
Bua bok	0·4 (0·4)	92·9 (7·1)	2·5 (2·5)
Cha phluu	0·2 (0·2)	90·7 (6·5)	100 (0·0)
Kra thin	0·6 (0·4)	88·8 (9·8)	0·0 (0·0)
Fak thong	0·7 (0·3)	88·9 (4·7)	0·7 (0·7)
Cha muang	0·2 (0·1)	67·1 (16·7)	0·0 (0·0)
Kheelek	0·2 (0·1)	53·2 (18·2)	0·0 (0·0)
Nieng	1·3 (0·1)	53·2 (14·8)	15·7 (10·2)
Ma kok	0·8 (0·5)	37·3 (16·1)	0·0 (0·0)
Mamuang himmaphaan	0·4 (0·3)	24·2 (12·3)	5·3 (5·3)
Nieng (germinating)	1·5 (0·3)	15·4 (8·1)	8·3 (8·0)
Rieng	0·4 (0·2)	5·0 (5·0)	4·7 (2·7)
Sato	2·8 (0·8)	2·3 (1·6)	7·8 (5·5)

[a]Mean values of endogenous free-form thiamin content and activity of antithiamin factors at 3 h incubation are presented together with their standard errors in brackets. The mean is the average value obtained from three lots of vegetable. Duplicate samples of freshly prepared crude extract of vegetable were analyzed.

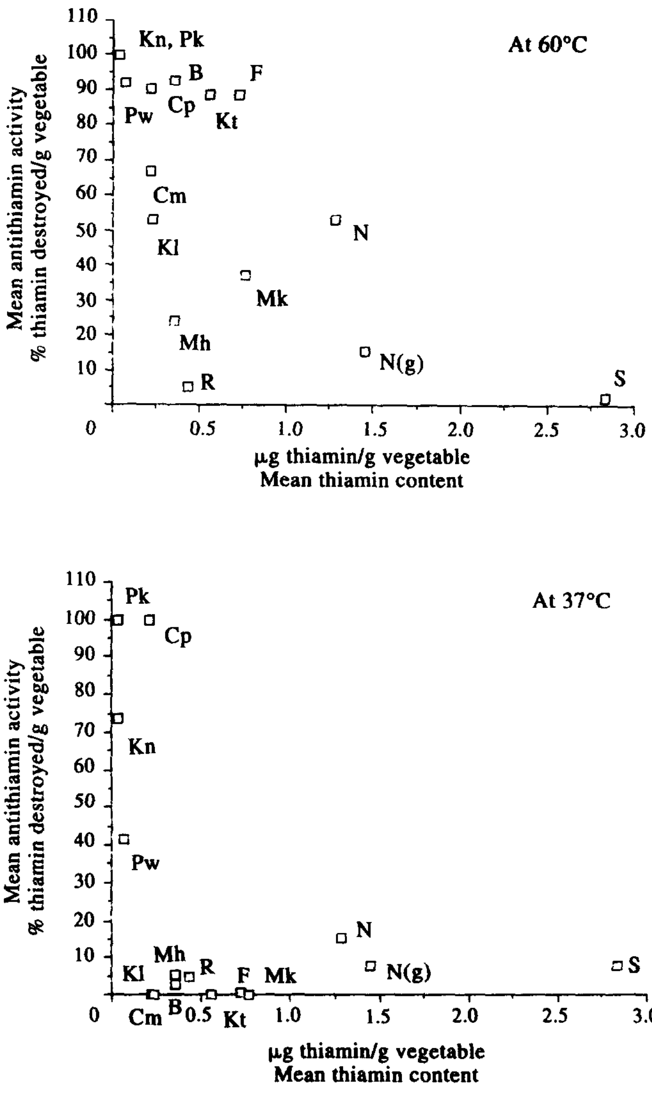

Fig. 1. Scatter plot of mean endogenous free-form thiamin content against mean activity of antithiamin factors in assayed vegetables at 60°C and 37°C for 3 h incubation.

Yai hospital, 26 infants (8·9%) were classified as low birthweight (birthweight less than 2500 g) (Mo-suwan, 1989). These data reflect the low energy and protein consumption of southern women. Furthermore, the main protein source in the area is fresh or fermented fish. It is well-known that fermented fish sampled from the north-east contains a high activity of heat-labile antithiamin factor. Fermented fish such as those made from fish guts showed 36·6 ± 7·2% thiamin destroyed/g food sample (unpublished data of the author). This information provides evidence that foods in the diet of southern people are not good sources of thiamin. Generally, Thai people consume an average of 100–150 g vegetables per person per day (Nakornchai *et al.*, 1975). Among vegetables consumed in the south, sato, rieng, nieng (germinating), mamuang himmaphaan and nieng figure prominently in the traditional diet of the region. Their ATF values at 60°C were 2·3, 5·0, 15·4, 24·2 and 53·2% respectively, and at 37°C 7·8, 4·7, 8·3, 5·3, 15·7%, respectively. In a recent epidemiological study of oesophagael cancer in southern Thailand, out of 404 non-diseased subjects 45% reported eating sato and 25% reported eating nieng at least as frequently as once a week (Chanvitan *et al.*, 1991). Such consumption of vegetables containing antithiamin factors would possibly contribute to poor thiamin status among the population. Further dietary survey and study of the effect of vegetable consumption on thiamin status are necessary to confirm an association.

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