Original Article

Evaluation of the significance of dietary folate from wild vegetables in Vietnam

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Data on the overall dietary folate intakes among high-risk groups in poor countries is very limited. Vegetables are considered good sources but the evaluation of their contribution is hampered by the lack of data on folate concentrations in many traditional foods. Data on the analysis of folate concentrations in 16 wild vegetables used in the Mekong Delta and the Central Highlands in Vietnam and an evaluation of the relative importance of different foods in folate intakes of women is presented. Vegetable samples were collected in four study villages, blanched and frozen samples were transported to Sweden for analysis. Freeze-dried samples were analysed for total folate quantification using a commercial radio protein binding assay. Daily folate intakes among women were estimated from 7-day food frequency interviews with 213 women. The folate concentration in the vegetable samples ranged from 10 to 96 µg/100 g. The mean estimated daily folate intake among the 213 women in the study areas was 251 µg. Vegetables contributed approximately one-third of the daily folate intake, of which 72% and 42%, respectively, in the two regions was from wild vegetables. A majority of the women (87%) got some dietary folate from wild vegetables and nearly one-third had mean daily folate intakes of > 50 µg from such hidden food sources. The evaluation of dietary folate is complicated by data gaps in food composition tables, the unreliability of existing food data, variations between methods used for folate analysis and limited understanding of the bioavailability of food folate.

Key words: analysis, Central Highlands, dietary folate intake, folate sources, Mekong Delta, radio-binding protein assay, Vietnam, wild vegetables, women.

Introduction

Folates and folic acid have received considerable attention in recent years. This group of B-vitamins takes part in carbon transfer reactions within the cell and has many regulatory mechanisms. Recent research has connected low folate status with a number of health risks.[1] In the general population low folate status has been associated with high homocysteine levels and increased risk of cardiovascular diseases, and has also been implicated in some forms of cancer.[2,3] Among women of child-bearing age, preconception supplementation with folic acid has been shown to reduce the occurrence of neural tube defects.[4] Low concentrations of folate in serum and diets during pregnancy have also been associated with increased risks of foetal growth retardation, preterm births and low birthweights.[5,6]

The realisation of the importance of adequate folate nutrition has led to substantial increases in the international recommended intake of folate.[7] Iron-folate supplementation of pregnant and lactating women and of adolescents girls is also becoming increasingly common.[8–11]

Folate is present in many different types of food, but legumes, green leafy vegetables and many fruits are considered rich sources. In Sweden, potatoes, vegetables and fruits contribute around 40% of the total dietary folate, while in some other western countries approximately 15–20% of the folate intake comes from potatoes, vegetables and bread.[12,13] Data on the relative importance of different foods in folate intake among high-risk groups in low income countries is very limited and such analysis is hampered by the fragmented data on folate concentrations in local food composition tables.

Data on folate intakes among women in Vietnam is generally lacking. In the national food composition tables the folate content is indicated only for a limited number of foods, using external references.[14–16] In connection with our research on the contribution of wild vegetables to micronutrient intakes among women we have therefore analysed the folate content of selected wild vegetables. The aim of this paper is to present these data and to raise some issues related to the evaluation of the significance of wild vegetables as sources of dietary folate in Vietnam. Data on the adequacy of dietary intake, the nutrient contribution of wild vegetables and nutrition status among the study population have been published elsewhere.[17,18]

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Materials and methods

Collection of vegetable samples

Samples of 16 species of vegetables were collected from two study villages in the Mekong Delta and two villages in the Central Highlands of Vietnam following the procedures suggested by Greenfield & Southgate.[19] In each of the study villages a random sample of 10 households was drawn from the study population that had previously participated in dietary surveys (BM Ogle, HTA Dao, Mulokozi G, Hambraeus, unpubl. data, 2000). Samples were gathered in the household surroundings in the mornings, placed in rice sacs in individual plastic bags and transported to the university laboratory for cleaning and weighing. After weighing, the samples were combined into a composite sample of approximately 1 kg from which a subsample of 100–200 g was taken. The subsamples were washed, blanched in boiling water for 1–2 min, deep-frozen and transported to Sweden for freeze-drying prior to folate analysis at the Swedish University of Agricultural Sciences, Uppsala.

Folate analysis

Freeze-dried samples of the plant foods were analysed for total folate quantification using a commercial Radio Protein Binding Assay (RPBA) kit (ICN Pharmaceuticals, CA, USA) originally aimed for clinical samples. This assay has been adjusted, optimised and validated for food samples (L Johannesson, K Forssén, C Witthöft, M Jägerstad, unpubl. data, 2001).[20] Freeze-dried samples (0.2 g) were extracted in duplicates in a boiling water bath (10 min) in 25 g of freshly prepared phosphate buffer pH 6.1, containing 1% ascorbic acid and 0.1% 2-mercaptoethanol.[21] After cooling to room temperature the samples were centrifuged (24000 *g*,15 min at 4°C) and 0.5 mL of chicken pancreas suspension (5 mg/mL; Difco, Detroit, USA) was added to a 1 mL extract and the samples were deconjugated for 3 h at 37°C. The enzyme reaction was inhibited by heat for 5 min in a boiling water bath. Following centrifugation (2000 *g*, 10 min at 4°C) the samples were subjected to the optimised commercial RPBA-kit which was used according to the instruction of the kit supplier. Calibrated 5-methyl-THF was used as an external standard. The relative radioactivity in the pellets was measured with the Cobra Auto Gamma scintillation counter (Packard-Acanberra Company, Meriden, USA). Plotting of standard curves and calculations of folate concentrations in the samples were performed using the computer programme, MultiCalc Routine (Wallac, Turku, Finland). To protect the folates from oxidation, all samples were kept under nitrogen atmosphere on ice during the preparation and the whole analysis was carried out in subdued lighting. All reagents used were of analytical purity and the water of Milli-Q grade or equivalent. Freeze-dried parsley was used as an in-house quality control. The RPBA was successfully used when analysing folate content in freeze-dried parsley. The parameter reproducibility and recovery controlled this (L Johannesson, K Forssén, C Witthöft, M Jägerstad, unpubl. data, 2001). Folate recovery of the sample pretreatment and quantification varied between 69 and 98% and the reproducibility (CV%) was always below 11%.

Study population and dietary assessment

Ethical permission was granted by the Research and Ethics Committee of the Faculty of Medicine, Uppsala University. In Vietnam local permissions for the field studies was obtained through the University of Can Tho and the University of Hue.

Formal dietary assessment surveys were carried out in the four study villages during the rainy seasons of 1997–98. In total, 110 and 103 women in the age range 19–60 years were interviewed in the Mekong Delta and the Central Highlands, respectively, using a 7-day frequency recall methodology (7dFFQ), quantified by usual portions. The study population, methodology and adequacy of dietary intake data have been published elsewhere.[17,18] Key features of the overall food intake in the study population are summarised in Table 1.

Data analysis

Daily folate intakes were estimated from the 7dFFQ using Ebis for Windows 95/98NT. A combination of folate values from the Vietnamese food composition tables and data from chemical analyses done specifically for this study were used for the estimation.[14] As all table values were for raw foods, a loss of folate in preparation was estimated, as suggested by McCance and Widdowson.[22] Thus 50% of the raw values for rice, cassava, sweet potatoes, cultivated vegetables, meat, fish and legumes were used. In the absence of national recommendations for folate intake in Vietnam, the current international recommended dietary allowance (RDA) for women has been used.[7] Statistical Packages for Social Sciences (SPSS 10.0) and Microsoft Excel 97 were used in the calculation of the dietary folate intake and the contribution of wild vegetables to overall intake.

Results and discussion

Folate concentrations in Vietnamese vegetables

The results from the folate analysis of blanched samples of 16 species of gathered vegetables are presented in Table 2. Four species had concentrations in the range of 50–100 µg folate while the remaining 12 species had lower concentrations. With the exception of *Nymphea lotus* and *Diplazium esculentum* all species were green leafy vegetables, but they differed with respect to the proportion of stem included in the edible portion.

Green leafy vegetables in general are considered good sources of folate. A concentration of >100 µg folate/100 g edible portion of uncooked vegetable or fruit is generally seen as a folate-rich food source, while 50–100 µg/100 g

Table 1. Key features of consumption of major foods of the study population (from Ogle *et al.*, 2000)

Food group	Mekong (n = 110) Mean intake†	Central Highlands (n = 103) Mean intake†
Rice	450	206
Tubers	106	
Cassava	–	487
Vegetables	274	268
Fruits	113	235
Fish	107	47
Meats	36	23
Beans/peas/nuts	2	6
Fats/oil	8	10

† values refer to g/person per day.

edible portion is considered a good source.[21] Vegetables are important in the Vietnamese diet, but the available data on the folate content of popular vegetables are old and data on wild vegetables (*rau dai*) are missing.[14] All data in the local food composition tables are also for uncooked foods. For comparison we have reviewed folate content in several other food composition tables with respect to key foods used in our study areas (Table 3).[22–27] This illustrates wide disparities between reported folate concentrations and differences in the reported losses in cooking. Most vegetables are eaten in their cooked form and folates are sensitive to oxidation and losses in heating, mostly through leaching. We have only been able to compare our data for blanched vegetables with raw values for three species, but taking into consideration losses in heating, our data with folate concentrations of 10–96 µg/100 g of blanched vegetable, fall in a relatively typical range for vegetables.

Table 2. Folate concentration of selected wild vegetables in Vietnam

Scientific name	Local name	Moisture percentage	Mean value *µg/100 g (CV%) $n = 4$
Terrestrial plants			
Sauropus androgynus	*Bô ngót*	88.3	96 (9)
Passiflora foetida	*Nhañ lông*	90.5	91 (12)
Plantago major	*Mã dê*	88.3	50 (7)
Centella asiatica	*Rau ma*	90.4	39 (6)
Basella rubra	*Mong toi*	93.0	36 (11)
Asystasia gangetica	*Huyet bo*	91.9	30 (5)
Houttuynia cordata	*La diep ca*	90.3	29 (35)
Portulaca oleracea	*Rau sam*	92.2	22 (7)
Bocopa monnieri	*Rau dang*	93.5	18 (7)
Commelina communis	*Rau trai*	91.8	16 (9)
Piper sarmentosum	*La lot*	87.0	15 (9)
Aquatic plants			
Nasturdium officinale	*Xa lach song*	94.2	64 (4)
Nymphea lotus, stem	*Bong song*	97.8	22 (4)
Ipomoea aquatica	*Rau muong*	91.8	20 (10)
Limnocharis flara	*Keo neo*	95.0	10 (10)
Forest plant			
Diplazium esculentum	*Rau ron*	92.3	27 (9)

Edible part - values expressed as µg/100 g of blanched deep frozen samples.

Table 3. Total folate content of selected foods (µg/100 g). A comparison of published values

	NIN Vietnam	USDA[3]	International minifood list[4]	McCance and Widdowson[5]	Our analysis[†]
Rice, white, raw	29[1]	9	6	20	NA
cooked	NA	4	NA		
Cassava, raw	24	27	15	19	NA
cooked	NA	11	NA		
Sweet potato, raw	52[1]	14	18	17	NA
cooked	NA	11	8	NA	
Guava, raw	170[1]	14	14	N	NA
Papaya, raw	1[1]	38	118	1	NA
Banana, raw	22[2]	19	19		
Watercress,raw	NA	9	104	N	64[†]
Sweet potato leaf, raw	88[1]	80	NA	NA	NA
Amaranthus sp. leaf	85[1]	85	104	85	NA
Mustard leaf	NA	187	NA	N	NA
Ipomoea aquatica	122[1]	114	NA		20[†]
Basella rubra	134[1]	140[‡]	NA		36[†]

[†]all blanched values; [‡]*Basella alba*; NA, not analysed; N, the nutrient is present in significant amounts but reliable information is lacking; NIN, National Institute of Nutrition.

Sources:[1] FAO Food composition tables for use in East Asia, 1972 in Food products in Vietnam, Composition and nutritive value 1994, National Institute of Nutrition

[2] Nutrient composition of foods, Ed Rastas Merja *et al.*, Helsinki, Finland 1989 in Food products in Vietnam, 1994, Composition and nutritive value 1994, National Institute of Nutrition

[3] USDA http://www.NAL.usda.gov/fnic/foodcomp/

[4] World Food Dietary Assessment System, International minifood list-Indonesia, http://otl.berkeley.edu/Worldfood.html

[5] McCance and Widdowson's The composition of foods, Fifth revised and extended edition. The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, UK.

Analysis of folate in foods, methodological issues

The RPBA was originally designed for analysing folate status in serum and blood, which mainly contain 5-methyl-THF. The assay is based on non-specific competitive binding of folates in the sample and radio-labelled folates in the kit for binding sites on folate binding proteins. Problems are caused because of varying affinity of the binder to different folate forms.[24] Thus the application of the RPBA might be restricted to food matrixes which mainly contain 5-methyl-THF, such as vegetables.[21]

The optimised RPBA was shown to be successful for analysing folate content in vegetables. Parsley, the in-house reference sample, showed a folate content close to the European food tables, thus proving the used methods of sample preparation and analysis to be reliable.[23] In spite of a more complex food matrix compared with serum, the CV(%) was similar and in accordance with the kit producer (i.e., 4,1–11,7).

Dietary folate intake among women in the study areas

The mean estimated daily folate intake among the 213 women in the study areas was 251 µg (Table 4). Mean intakes at the 25th and 75th percentile, respectively, were 164 µg and 313 µg. In the Mekong Delta and the Central Highlands populations, 46% and 30%, respectively, had intakes of < 200 µg/day. Only 11% of the women had intakes > 400 µg, which is the most recent recommendation.[7] These data compare relatively well to dietary assessments in European countries where many studies typically indicate a wide range in the dietary folate intakes among women. Mean daily intakes of 200–300 µg/day were common in European studies, when fortified foods and supplements were excluded.[12]

The major contributors to folate intake are shown in Fig. 1 a,b. Several interesting findings emerge. First, a high rate (44 and 46%) of the folate intake in the Mekong Delta and the Central Highlands, respectively, was derived from the staple foods (rice, cassava and sweet potatoes). Second, vegetables contributed approximately one-third of the daily folate intake in the two areas, of which 72 and 42%, respectively, was folate from wild vegetables. Women made use of a large variety of wild vegetables and the significance of this group of foods is also shown in Table 5, where the mean daily intakes of individual species of wild vegetables in the two study regions are shown. A majority of the women (87%) got some dietary folate from wild vegetables and nearly one-third had mean daily folate intakes of > 50 µg from such hidden food sources. A third important finding is the significant difference between the two regions in the categories of foods that were major contributors (Fig. 1). Cassava contributed one-fifth of the dietary folate in the Central Highlands and fruits, especially bananas and guavas were important contributors in that season. In the Central Highlands, the higher consumption of sweet potato leaves and mustard greens resulted in the larger share of cultivated vegetables to the overall folate intake. By contrast in the Mekong Delta, cultivated vegetables consisted more of vegetables such as radish, squash, cucumbers and carrots while most of the wild species were green leafy vegetables.

Table 4. Dietary folate intake among women in Vietnam

	Mekong Delta (n = 110)	Central Highlands (n = 103)
Mean energy intake MJ/d (kCal/day)	9.3 (2216 ± 679)	8.5 (2034 ± 535)
Mean total folate µg	235 ± 120	268 ± 118
% women < 300 µg/day	77	64
% women < 200 µg/day	46	30

Conclusions

In this paper we have illustrated that wild vegetables can make a significant contribution to dietary folate intakes and that it is important to be observant of this in dietary interviews. The evaluation of their role is however, complicated by data gaps in food composition tables, the unreliability of existing food data, variations between methods used for folate analysis and limited understanding of the bioavailability of food folate. Our overall conclusion is that the database for the folate concentrations of foods in Vietnam is extremely fragmented and that this makes it virtually impossible to assess dietary folate intakes unless food analyses are made simultaneously. All the factors above make it difficult for nutrition/health professionals in low-income countries such as Vietnam, to evaluate folate intakes with any certainty and to formulate public recommendations for dietary folate.

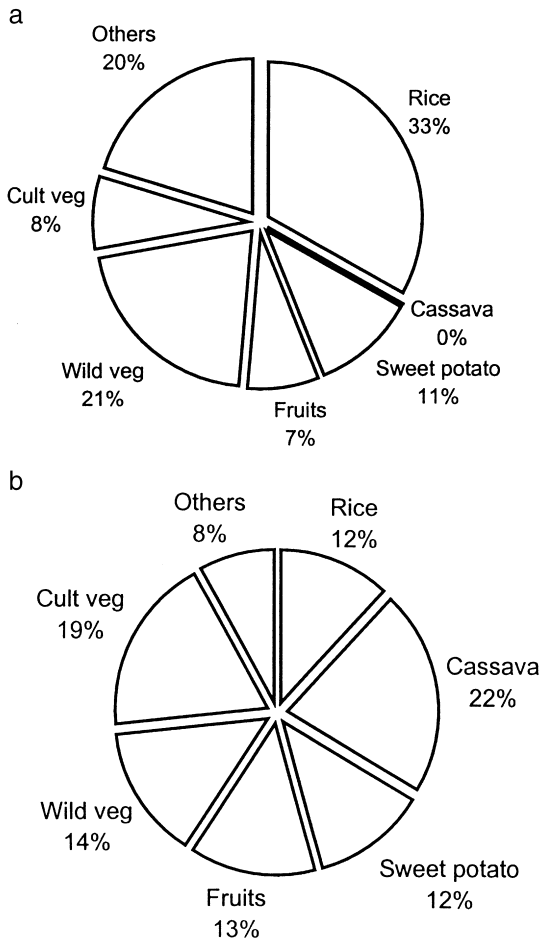

Figure 1. (a) Major dietary sources of folate. Mekong Delta population. Percentage distribution by food group. (b) Major dietary sources of folate. Central Highlands population. Percentage contribution by food group.

Table 5. Examples on mean daily intakes of individual species of wild vegetable among women in the study villages (from Ogle *et al.*, 2000)

Scientific name	7dFFQ Mekong Delta Mean daily intake g (%consuming)		7dFFQ Central Highland Mean daily intake g (%consuming)
	Flood period	Rainy season	Rainy season
Aquatic plants			
Eleocharis spp.	79 (45)	122 (44)	
Ipomoea aquatica†	28 (37)	58 (61)	63 (26)
Limnocharis flara	82 (31)	58 (25)	
Nasturdium officinale			38 (39)
Nymphea lotus	91 (55)	57 (25)	
Terrestrial plants			
Alternanthera repens		21 (5)	
Basella rubra§		61 (8)	18 (20)
Centella asiatica	21 (28)	35 (41)	32 (51)
Commelina communis		32 (20)	
Gynura crepidioides			29 (5)
Homalonema occulta			53 (24)
Houttuynia cordata			17 (24)
Passiflora foetida		25 (19)	
Portulaca oleraceae			18 (19)
Sauropus androgyn‡	21 (30)	27 (45)	28 (5)
Schismatoglottis calyp			60 (18)
Schizostschyum avicul			57 (28)

† cultivated by approximately one-third of households in Mekong Delta and mostly purchased in Highlands.
‡ mostly cultivated in Mekong sites, yet grouped with gathered plants by women.
§ often cultivated yet listed as *rau dai*.

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